PREVALENCE OF DENGUE VIRAL INFECTIONS AMONG FEBRILE PATIENTS
IN MOMBASA COUNTY, KENYA

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of Master
of Science Degree in Infectious Diseases in the School of Medicine of
Kenyatta University

June, 2014
DECLARATION
This thesis is my original work and has not been presented for a degree or other awards in any other University.

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DEDICATION

I dedicate this thesis to my parents, Mr. Sibabi Nyukuri and Mrs. Dinnah Nekesa. The person I am today is a direct result of my parent’s unconditional love. Their support and advice have always been part of my decision process and I am a better man for it. With all of my heartfelt sentiment, I wish them many years of happiness.
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God bless you.
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<td>CPGH</td>
<td>Coast Provincial General Hospital</td>
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<tr>
<td>DENV</td>
<td>Dengue Virus</td>
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<td>DF</td>
<td>Dengue Fever</td>
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<td>DHF</td>
<td>Dengue Hemorrhagic Fever</td>
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<td>DSS</td>
<td>Dengue Shock Syndrome</td>
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<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
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<td>Flavi</td>
<td>Flavivirus</td>
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<tr>
<td>GMT</td>
<td>Geometric Mean Titer</td>
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<tr>
<td>ICTV</td>
<td>International Committee on Taxonomy of Viruses</td>
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<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
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<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
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<tr>
<td>JEV</td>
<td>Japanese Encephalitis Virus</td>
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<tr>
<td>KEMRI PD</td>
<td>Kenya Medical Research Institute Production Department</td>
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<tr>
<td>rpm</td>
<td>revolutions per minute</td>
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<tr>
<td>UTR</td>
<td>Untranslated Regions</td>
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<td>WHO</td>
<td>World Health Organization</td>
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ABSTRACT
Dengue virus infection is one of the major global public health problems. It is caused by one of the four dengue virus (DENV) serotypes that are transmitted by *Aedes* mosquitoes. Following infection, an individual remains vulnerable to re-infection with a different serotype of the DENV. The infection usually occurs with clinical manifestations ranging from an asymptomatic or mild febrile illness as classical dengue fever to the potentially life-threatening illness, dengue hemorrhagic fever and dengue shock syndrome. Despite the public health relevance, prevalence of DENV infections among febrile patients in Mombasa County is unknown. This study was conducted from February 2012 to July 2012 among patients visiting Coast Province General Hospital with high fever. The study was aimed at determining the prevalence of DENV infection among febrile patients and describe the month-wise trend of the disease. A total of 390 blood serum samples were collected and DENV specific IgM and flavivirus IgG antibodies were determined by in-house enzyme linked immunosorbent assay (ELISA). Out of 390 febrile cases, 54 (13.9%) were found to be positive for anti-DENV IgM. Among the 54 dengue positive cases, 37 (68.5 %) were primary DENV infection and 17 (31.5%) were secondary DENV infection. The most affected age group was 36-45 years (20.4%) and least affected group being 6-15years (8.3%). Prevalence in difference age groups was statistically significant (p = 0.021). Primary DENV infection was common among the age group between 36-45 years while secondary dengue affected mostly the age group 26-35 years. In terms of primary DENV infection against secondary DENV infection, it was observed that infants (<1 year) were the most affected but this was not statistically significant (p = 0.057). The relationship between gender and DENV infections was not statistically significant (p = 0.936). Although, females aged between 26-35 years (p = 0.010) and males aged above 46 years (p = 0.012) were the most affected with DENV infection. Month-wise distribution of DENV infection was observed in February (20.0%) with least occurrence in July (4.7%). The association between the month and occurrence of disease was not statistically significant (p = 0.325). The present study has reported 13.9% prevalence of Dengue virus infections as the cause of acute undifferentiated fever among febrile patients in Mombasa County. Thus, calls for government attention to develop resources at hospital laboratories for early dengue diagnosis and management of patients, coupled with general awareness among the public and constant vigilance by the health care officials could help in combating dengue.
CHAPTER ONE: INTRODUCTION

1.1 Background

Dengue virus (DENV) infection is one of the mosquito-borne viral diseases with a major impact on public health, globally (Guzman et al., 2010). World Health Organization (WHO) data suggest that at least 100 countries are endemic of Dengue virus transmission. About 3.5 billion people, 55% of the world’s population living in tropical and subtropical regions are at risk, with about 50 million DENV infections occurring annually and approximately 500,000 requiring hospitalization annually (WHO, 2009). The average case fatality rate is around 5%, and mainly among children and young adults (Beatty et al., 2007).

Dengue virus is a positive-sense, single-stranded RNA enveloped virus that comprises of four serotypes (DENV 1, 2, 3 and 4) that belong to family Flaviviridae and genus Flavivirus (ICTVdB, 2006). All four serotypes of DENV are serologically related, but antigenically distinct (Zanotto et al., 1996). They produce a spectrum of clinical illnesses ranging from a classical dengue fever (DF) to severe and potentially fatal complications known as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (WHO, 2009). Dengue fever is marked by a sudden onset of high fever, severe headache and retro-ocular pain and myalgia. The symptoms and signs may be very similar to other viral infections. The distinctive characteristics of DHF and DSS consist of hemorrhagic manifestations, plasma leakage, and profound shock. Antibody dependent enhancement (ADE) of viral replication is considered as a major reason for severity of DHF and DSS (Halstead, 2002). However, other factors also might be associated with DHF, such as DENV genotype polymorphisms in human leukocyte antigen (HLA) and other host genes (i.e. transporter associated with antigen processing (TAP) and human platelet antigen (HPA) (Vaughn et al., 2000; Soundravally and Hoti, 2007; Stephens, 2010).
Peak DENV infection occurs after period of increased rainfall due to increased multiplication of the mosquito vector, *Aedes aegypti* (*Ae. aegypti*) (El-Badry and Al-Ali, 2010). *Aedes* mosquitoes shelter indoors and bite during the daytime. They are adapted to breed around human dwellings, in water containers, vases, cans, tires, and other discarded objects (El-Badry and Al-Ali, 2010). *Ae. albopictus* is also the vector for DENV which contributes significantly to transmission in Asia and whose presence is spreading in Latin American countries (Roiz et al., 2008). Dengue outbreaks have also been attributed to *Ae. polynesiensis* and *Ae. scutellaris*, but to a lesser extent (Rodhain and Rosen, 1997). Early diagnosis of DENV infection is important for proper treatment of DHF and DSS to avoid fatal outcome. Currently, several dengue vaccine candidates are in an advanced stage of development (Morrison et al., 2010). For example, Sanofi Pasteur’s ChimeriVax-DENV vaccine has recently entered phase 3 clinical testing (Guy et al., 2010; Coller and Clements, 2011).

**1.2 Problem Statement**

Dengue virus infection is a complex disease with symptoms being difficult to distinguish from other common febrile illnesses during acute phase and can progress from a mild, non-specific viral disease to severe cases characterized by thrombocytopenia, hemorrhage manifestations and hemo-concentration due to plasma leakages. Majority of febrile illnesses in Mombasa County are treated as presumptive malaria, often without proper medical examination and a laboratory diagnosis. Therefore, many patients with fever are designated as having fever of unknown origin or malaria and remain without a laboratory diagnosis even if they fail to respond to antimalarial drugs. This situation is generally due to lack of affordable diagnostic reagents. The scenario indicates that many cases of DENV infections are undiagnosed or even misdiagnosed.
Additionally, presence of dengue vector *Aedes aegypti* in the coastal region of Kenya as reported by Mwangangi *et al.*, (2012). Individual exposure differences to dengue infective bites may be related to prevalence with specific demographic factors such as age and gender that have not been reported among febrile patients in the County of Mombasa.

**1.3 Justification of the Study**

Dengue virus serotypes 1, 2, and 3 have been identified and invariably caused outbreaks in eastern Africa region (Amarasinghe *et al.*, 2011). These outbreak reports highlighted the vulnerability of the Mombasa County to DENV transmission and their capacity for rapid expansion across the entire coastal region. Since, Mombasa County shares similar conditions as those of the neighboring countries or islands in eastern Africa region that favor the dengue transmission. These include environmental conditions that favor mosquito proliferation and interaction with humans, such as warm climate, high rainfall, and overcrowding. Other factors that may facilitate dengue transmission are presence of principle vector *Aedes aegypti* in the coastal region, inadequate and deteriorating public health infrastructure, and lack of effective mosquito control (Mwangangi *et al.*, 2012).

Additionally, outbreak of classical dengue fever was reported in the coastal towns of Malindi and Kilifi in 1982, with subsequent isolates of DENV-2 made from Mombasa and Diani showing a wide distribution of DENV infection along the Kenyan coast (Johnson *et al.*, 1982; Sang and Dunster, 2001). Since then sporadic cases of DENV infections have been ignored indicating potential endemicity of the dengue infection in the general population of county. This was a significant public health problem because of the clinical oversight and lack of appropriate laboratory diagnostic reagents. Implying that there was a potential risk for dengue cases as multiple serotypes could be circulating among the human population in the study
region putting them at risk of immune mediated DSS when previously infected persons become re-infected with a heterologous serotype (Gubler et al., 2002).

However, gender disparities affect the county of Mombasa with females main work being domestic. Hence, presence of a highly domesticated dengue vector *Aedes aegypti*, the females and pre-school children are at a higher risk of dengue infection as they spend most of their time at home. Therefore, it was important to understand the local prevalence of dengue infection in the county of Mombasa.

### 1.4 Significance of Study

Exposure to the dengue virus generally occurs in the infantile to juvenile period among residents in dengue endemic areas, and the prevalence of DENV infection increases with age and reaches its peak before adolescence. Collecting information on the prevalence among persons with febrile illness would be an initial step in determining the extent of dengue infections. This will help the physicians to consider possibility of dengue cases when handling febrile patients, thereby proper management of the dengue patient to avoid fatal complications. Dengue prevalence is usually attributed to gender related differences in exposures, as gender roles and exposures change over the human lifespan. Examining both age and gender will provide prevalence of dengue stratified data that will help on targeting specific preventive measures. Additionally, the study findings will deliver effective communication and coordination to the government and non-governmental partners, and the community to implement policy on adequate infection prevention practices and improve vector control programmes to reduce the dengue burden in the County.

### 1.5 Research Questions

i) What is the prevalence of DENV infections among febrile patients in Mombasa County?
ii) What age and gender is most affected by DENV infections among febrile patients in Mombasa County?

iii) What is the proportion of primary and secondary DENV infection among febrile patients in Mombasa County?

iv) Which month has the highest prevalence of dengue cases in Mombasa County?

1.6 Null Hypothesis

Dengue virus infection is not a health problem among febrile patients in Mombasa County.

1.7 General Objective

To determine the prevalence of DENV infection among febrile patients in Mombasa County.

1.8 Specific Objectives

i) To determine the prevalence of DENV infection by age and gender of among febrile patients in Mombasa County.

ii) To determine the proportion of primary and secondary DENV infection among febrile patients in Mombasa County.

iii) To determine the month-wise distribution of DENV infection among febrile patients in Mombasa County.
CHAPTER TWO: LITERATURE REVIEW

2.1 Dengue Viral Infection
Dengue virus (DENV) infection is an acute febrile illness, which occurs after an incubation of 4-10 days. Infection parity is known to be a critical factor of disease severity. Primary DENV infection with any of the four DENV serotypes is believed to elicit lifelong immunity against that serotype, but confers partial or transient immunity against other serotypes. Cross-reactive, but sub-neutralizing DENV-reactive IgG acquired by a previous heterotypic serotype infection may enhance DENV infectivity which may result in higher viral burden and contribute to induced disease severity. Heterologous secondary DENV infections have been associated with large, clinical outbreaks of Dengue hemorrhagic fever or Dengue shock syndrome (DHF/DSS), where severe dengue occurs most frequently in children (WHO, 1997).

2.2 Clinical Manifestations
Most DENV infections are asymptomatic, but may result in a wide spectrum of disease that differs in severity from mild undifferentiated fever, the classical DF (Guha-Sapir and Schimmer, 2005), to the potentially fatal complications known as DHF and DSS (Figure 2.1). Clinical presentation in both children and adults may vary in severity depending on the immune status, age and the genetic background of the patient (WHO, 2009).
2.2.1 Dengue Fever

Most patients display mild fever or remain asymptomatic. However, symptomatic infection presents as classic dengue fever (DF) with an incubation period of 4 to 10 days. The clinical features of DF frequently depend on the age of the patient (Hammond et al., 2005). Children are often asymptomatically infected with DENV but may demonstrate several clinical syndromes. Infants and young children most often present with an undifferentiated febrile illness accompanied by a maculopapular rash seen on the trunk and inside of the arms (George and Lum, 1997). Older children and adults typically present with classic DF characterized by an acute sudden onset saddleback fever, severe headache, nausea and vomiting, myalgia, retro-orbital pain, an early maculopapular rash, low grade thrombocytopenia and hepatomegaly (Henchal and Putnak, 1990). Patients with DF recover
in two to seven days and suffer no short- or long-term sequelae of illness. The virus disappear from bloodstream at approximately the same time that the fever dissipates (Rothman, 1999).

2.2.2 Dengue Hemorrhagic Fever

Dengue Hemorrhagic Fever (DHF) usually follows a secondary dengue infection. In infants, it may follow a primary infection due to maternally acquired dengue antibodies (Halstead et al., 2002). The clinical course of DHF is divided into three phases, namely, febrile, critical, and convalescent phases (Figure 2.2). The febrile phase begins with sudden onset of fever accompanied by generalized constitutional symptoms and facial flush. The fever is high grade (usually >38.5°C), intermittent, and associated with rigors. The fever lasts for 2-7 days and then falls to normal when the patient either recovers or progresses to the plasma leakage phase (CDC, 2012a; Srikitakhachorn et al., 2007).

Some patients remain ill despite normalization of temperature and therefore progresses to DHF. Onset of plasma leakage is characterized by tachycardia and hypotension. The patient sweats, becomes restless, and has cool extremities. In less severe cases, the changes are minimal and transient, reflecting a mild degree of plasma leakage. Most patients recover from this stage spontaneously or after a short period of fluid and electrolyte replacement. In severe cases with high plasma leakage, patients may develop full-blown circulatory shock characterized by prolonged capillary refill time and narrow pulse pressures (WHO, 2009).
Figure 2.2 Phases of infection resulting in DHF (CDC, 2012a)

During the phase of plasma leakage, pleural effusions and ascites are common. Pericardial effusions may also be seen. Myocarditis is associated with increased morbidity and mortality.

Fever and hemo-concentration due to plasma leakage is most commonly observed before the subsidence of fever and the onset of shock (Kalayanarooj et al., 2002).
2.2.3 Dengue Shock Syndrome

Dengue shock syndrome (DSS) is associated with almost 50% mortality. After a certain level of plasma leakage, the compensatory mechanisms become insufficient and blood pressure drops rapidly. Pulse pressure drops below 20 mmHg and symptoms of hypovolemic shock develop; sudden collapse, cool clammy skin, rapid weak pulse, circumoral, easy bruising and bleeding (hematemesis, melena, epistaxis), and myocarditis. Warning signs include severe abdominal pain, vomiting, irritability and somnolence, fall in body temperature and severe thrombocytopenia (Gibbons and Vaughn, 2002). Patients die from multi-organ failure and disseminated intravascular coagulation. Most patients remain fully conscious to the terminal stage. The duration of shock is short and the patient rapidly recovers with appropriate supportive therapy. DSS may be accompanied by encephalopathy caused by metabolic and electrolyte disturbances (Gurugama et al., 2010).

2.3 Dengue Case Classification

2.3.1 Dengue Case Classification

World Health Organisation devised a formal classification scheme that defined dengue as either asymptomatic, DF or DHF/DSS. The DHF category is further classified based on the degree of haemorrhagic manifestations and plasma into four grades of severity (Figure 2.3).
Dengue Hemorrhagic Fever (DHF) grades III and IV can lead to DSS and total fatal outcome. DHF grade I is characterized by a severe fever with non-specific constitutional symptoms. The only required manifestation of hemorrhage is a positive tourniquet test. A patient is diagnosed as DHF grade II if they exhibit spontaneous bleeding in addition to a positive tourniquet test or easy bruising such as melena and hematemesis, epistaxis, gingival bleeding, gastro intestinal bleeding, hematuria and menorrhagia. Patients receive the more serious diagnosis as DHF grade III and IV if they exhibit signs of circulatory failure and massive bleeding. DSS is sometimes observed among DHF grade III and IV if they exhibit profound hemorrhage.
shock characterized by an undetectable blood pressure or pulse. However, in the absence of rash and thrombocytopenia signs of hemo-concentration and vascular permeability, these patients were not to be diagnosed with DHF. Instead, they were assigned the diagnosis of DF with hemorrhage (WHO, 1997).

Many physicians treating dengue patients criticized the WHO 1997 diagnostic criteria because of its rigidity and difficulties of application in clinical practice (Deen et al., 2006). In a number of retrospective chart studies, the sensitivity of the WHO 1997 diagnostic criteria approached only 80%, suggesting that a vast number of DHF/DSS cases are under-diagnosed and under-reported (Rigau-Perez, 2006). Firstly, there were many case reports of patients with severe dengue with shock who did not fulfill all the 4 grades of DHF (Figure 2.3). These patients would be classified as dengue fever, if the WHO criteria were to be strictly applied. Secondly, patients with severe organ impairment such as liver, respiratory, cardiac and brain dysfunction were not captured as having severe disease based on the previous classification. Lastly, the requirement of 20% increase in hematocrit (HCT) as one of the evidence of plasma leakage was difficult to fulfill since the baseline HCT was not available in most patients and therefore, the interpretation of plasma leak was only retrospectively and early fluid administration could affect the level of HCT.

2.3.2 Revised Dengue Case Classification

In 2006, WHO Dengue Scientific Working Group recommended additional research into dengue diagnostics and triaging of patients for optimized clinical management. Further studies on the use of clinical guidelines for dengue diagnosis, including the Dengue Control (DENCO) study, led to the re-classification of dengue according to levels of severity as having dengue without warning signs, dengue with warning signs, and severe dengue based on clinical manifestations with or without laboratory parameters (Figure 2.4). Patients who
recover following defervescence are considered to have non-severe dengue, but those who deteriorate tend to manifest warning signs. However, further deterioration is classified as severe dengue, though recovery is possible if appropriate and timely treatment is given (WHO, 2009).

**Figure 2.4. Criteria for dengue and severe dengue.** Dengue fever cases can be classified as probable dengue or dengue with warning signs. The presence of warning signs indicates that the patients will require strict observation and medical intervention as these patients are likely to develop into severe dengue cases. Those without warning signs may, however, also develop into severe dengue cases. Severe dengue cases are characterized by severe plasma leakage, severe bleeding or severe organ impairment (WHO, 2009).
2.4 Transmission of Dengue Virus Infection

2.4.1 Mosquito Vectors

All the known vectors of DENV are mosquitoes belonging to genus Aedes (Ae.), subgenus Stegomyia (Figure 2.5). The species involved in transmission include Ae. aegypti usually in an urban environment and globally exists in tropical area. However, Ae. albopictus is present in Asia and the pacific. Ae. polynesiensis only exists in the Pacific (Rodhain and Rosen, 1997).

![Ae. aegypti and Ae. albopictus](image)

Figure 2.5 Mosquito vectors for DENV transmission (Rodhain and Rosen, 1997)

The life cycle of a mosquito consists of four separate stages: egg, larva, pupa and adult (Figure 2.6), the first three stages requiring an aqueous environment. The duration of the developmental stages depend on the environment’s temperature, water and availability of food at the larval stage. For Ae. aegypti, it takes 8-10 days at room temperature (Gubler, 1997). Adult male mosquito feed on flower nectar and juices of fruits for flight energy. The female requires a blood meal for egg development. Human blood is preferred and the ankle area is a favoured feeding site (Monath, 1994).
Figure 2.6 Life cycle of *Aedes* mosquito (Wongkoon et al., 2007)

*Aedes aegypti* female mosquito is highly anthropophilic (Huber et al., 2008) and prefers to feed during the day - two hours after sunrise and few hours before sunset is the most appropriate time, although they feed all day indoors and on overcast days. Female *Ae. aegypti* mosquito shows a preference for laying their eggs in domestic containers, but may also use rainwater-accumulating containers present in peridomestic environments (Wongkoon *et al.*, 2007; El-Badry and Al-Ali, 2010). Its adaptation to human habitats and its desiccation-resistant eggs have allowed it to flourish in urban centers. They have a life span of 8 to 15 days and flight range for females is about 30 to 50 meters per day. These mosquitoes are unique in that they feed on more than one person per gonadotrophic cycle and will resume feeding on a second individual if interrupted (El-Badry and Al-Ali, 2010).
2.4.2 Dengue Virus Transmission Cycles

Two transmission cycles are known for DENV, one of them involving non-human primates (monkeys) and jungle mosquitoes, referred to as the sylvatic cycle, and the second being the urban cycle that involves *Ae. aegypti* - human - *Ae. aegypti* which is most important transmission cycle that causes huge outbreaks in the tropics (Gubler and Meltzer, 1999) (Figure 2.7).

The life cycle of DENV involves a replication step in both mosquito and human hosts. Infected humans are the main carriers and multipliers of the virus, serving as a source of the virus for uninfected mosquitoes (Monath, 1994). The virus circulates in the blood of infected humans for two to seven days and at approximately the same time patient develop fever. Uninfected *Aedes* mosquitoes acquire the virus when they feed on an individual during this period (Monath, 1994).

![Figure 2.7 Transmission cycle of DENV (Whitehead et al., 2007)](image)

Once a mosquito has fed on a viremic human, the virus replicates in the arthropod mid-gut and disseminates to the salivary glands within 8-12 days. Following dissemination to the
salivary glands, female *Aedes* mosquitoes are able to transmit DENV to new hosts. However, for the virus infection to be sustained in the vector mosquito, virus titer in the human host should exceed $10^5 – 10^7$ virus particles per ml (Monath, 1994). The vector itself is thought to function as an important biological filter for maintaining the virus titers at high level (Monath, 1994). In periods of low virus transmission, the DENV may survive through transovarial transmission from parent to progeny and possibly also between mosquitoes sexually (Khin and Khan, 1983).

Direct person-to-person transmission has not been documented. Although, a few case reports have been published on transmission of DENV through exposure to DENV-infected blood, organs, or other tissues from blood transfusions, solid organ or bone marrow transplants, percutaneous and mucous membrane contact with dengue-infected blood (De Wazieres *et al.*, 1998; Chen and Wilson, 2004; Tan *et al.*, 2005; Wilder-Smith *et al.*, 2009).

### 2.5 Factors Influencing DENV Transmission

Many factors contribute to the emergence and sustained transmission of DENV. Uncontrolled urbanization, expanding urban population, poverty, ineffective public health infrastructure, faster modes of transportation, globalization of trade and increased international travel have all been implicated as factors leading to the spread of dengue around the world (Gubler and Clark, 1995). Rapid urbanization is probably the single most important contributing factor especially where the resulting populated centers tend to lack piped water and residents have to resort to using containers to store water that often end up as breeding sites for the *Ae. aegypti* vector. The lack of adequate sewage systems often leads to the same result.

Relaxation of vector control efforts, expansion of the vector range, and the build-up of vector resistance to insecticides are some of the recognised factors affecting the contribution of the mosquito vector (Gubler and Clark, 1995). The impact of environmental factors on the
transmission and spread of mosquito-borne diseases as exemplified by effects of temperature, rainfall and humidity on vector transmission cycles are also well known (Nakhapakorn and Tripathi, 2005). Besides the effect of generalised climatic factors (global warming, for example) the local ecology probably plays an equal, if not more important, role in a disease as complex as dengue (Reiter, 2001; Johansson et al., 2009). Inherent differences in the virulence of the introduced DENV strains have also been suggested as being a contributing factor in causing outbreaks and in the emergence of the severe form of dengue disease (Rodhain and Rosen, 1997).

2.6 Global Geographical Distribution

Dengue virus is the world’s most geographically widespread arthropod-borne virus and its geographical distribution is inherently tied to the range and habitat of its principal vector mosquitoes. Dengue infections are reported in more than one hundred tropical and sub-tropical countries worldwide, mostly in urban and semi-urban areas where the vectors are widely found (WHO, 2007a). Dengue is hyperendemic in many of these urban centers with co-circulation of multiple DENV serotypes. In non-tropical regions, dengue is usually the result of infection from international travelers that have visited dengue-endemic areas (WHO, 2007a). The larvae of the principal vector *Ae. aegypti* under naturally changing temperature are capable of developing into adults in conditions lower than 10°C, whereas those of *Ae. albopictus* can survive even lower temperatures. As shown in Figure 2.8, the southern parts of the United States and Europe, and major parts of Australia and Africa are among the endemic areas of dengue transmissions.

In Africa, DENV transmission is endemic to 34 countries. Local transmissions have been reported in 22 countries, with most transmission occurring in Eastern Africa (Table 2.1) (Amarasinghe et al., 2011). Nearly 300,000 cases were reported in 5 large epidemics in the
Seychelles (1977–1979), Réunion Island (1977–1978), Djibouti (1992–1993), Comoros (1992–1993), and Cape Verde (2009). In the remaining 12 countries, dengue was diagnosed only for travelers returning to countries to which dengue was not endemic but never reported as occurring locally in these 12 countries (Table 2.1) (Amarasinghe et al., 2011).

Dengue virus transmission in Kenya dates back to 1982 when an outbreak of DENV 2 was reported in the coastal towns of Malindi and Kilifi (Johnson et al., 1982). Additional isolates of DENV 2 were made from Mombasa and Diani showing a wide distribution of DENV infection along the Kenyan coast (Sang and Dunster, 2001). However, all the four DENV serotypes have been isolated in Africa. DENV-2 has been reported to cause most epidemics, followed by DENV-1 (Amarasinghe et al., 2011).

Figure 2.8 Countries or areas with active dengue transmission and principal vector *Ae. aegypti* (CDC, 2012a). Light-coloured (yellow) areas indicate dengue endemic countries and areas
Table 2.1 Countries in Africa with evidence of DENV transmission

<table>
<thead>
<tr>
<th>Type and country</th>
<th>Year</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Locally acquired, n = 7</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cape Verde</td>
<td>2009†</td>
<td>3</td>
</tr>
<tr>
<td>Egypt</td>
<td>1779, 1887, 1927</td>
<td>Unknown</td>
</tr>
<tr>
<td>Eritrea</td>
<td>2005</td>
<td>Unknown</td>
</tr>
<tr>
<td>Mauritius</td>
<td>2009</td>
<td>Unknown</td>
</tr>
<tr>
<td>Réunion</td>
<td>1977–1978†</td>
<td>2</td>
</tr>
<tr>
<td>Seychelles</td>
<td>1977–1979†</td>
<td>2</td>
</tr>
<tr>
<td>Sudan</td>
<td>1984–1986</td>
<td>1 and 2</td>
</tr>
<tr>
<td><strong>Locally and travel acquired, n = 15</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angola</td>
<td>1986, 1999–2002‡</td>
<td>Unknown</td>
</tr>
<tr>
<td>Burkina faso</td>
<td>1925</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>1983–1986</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2003–2004, 2007‡</td>
<td>Unknown</td>
</tr>
<tr>
<td>Comoros</td>
<td>1943–1948</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>1984, 1992–1993‡</td>
<td>Unknown</td>
</tr>
<tr>
<td>Djibouti</td>
<td>1991–1992†</td>
<td>2</td>
</tr>
<tr>
<td>Côte d’Ivoire</td>
<td>1982</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1998</td>
<td>1</td>
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<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>3</td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
<td>2002–2005</td>
<td>2</td>
</tr>
<tr>
<td>Kenya</td>
<td>1982</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1984–1986</td>
<td>2</td>
</tr>
<tr>
<td>Madagascar</td>
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</tr>
<tr>
<td></td>
<td>2006</td>
<td>1</td>
</tr>
<tr>
<td>Mozambique</td>
<td>1984–1985†</td>
<td>3</td>
</tr>
<tr>
<td>Nigeria</td>
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<td>1</td>
</tr>
<tr>
<td>Senegal</td>
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</tr>
<tr>
<td></td>
<td>1979</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1980–1985</td>
<td>2 and 4</td>
</tr>
<tr>
<td></td>
<td>1990, 1999</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2007‡</td>
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</tr>
<tr>
<td></td>
<td>2009</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1992–1993</td>
<td>2 and 3</td>
</tr>
<tr>
<td>South Africa</td>
<td>1927†</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2006‡, 2010‡</td>
<td>3</td>
</tr>
<tr>
<td>Zanzibar</td>
<td>1823, 1870, 2010‡</td>
<td>Unknown</td>
</tr>
<tr>
<td><strong>Travel/expatriate acquired, n = 12</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benin</td>
<td>1987–1993§</td>
<td>Unknown</td>
</tr>
<tr>
<td>Equatorial Guinea</td>
<td>1999–2002</td>
<td>Unknown</td>
</tr>
<tr>
<td>Gabon</td>
<td>1999–2002†</td>
<td>Unknown</td>
</tr>
<tr>
<td>Mali</td>
<td>2008</td>
<td>2</td>
</tr>
<tr>
<td>Namibia</td>
<td>1999–2002, 2006‡</td>
<td>Unknown</td>
</tr>
<tr>
<td>Rwanda</td>
<td>1987–1993§</td>
<td>Unknown</td>
</tr>
<tr>
<td>Zambia</td>
<td>1987–1993§</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

†Large local outbreaks.
‡TropNet Europ Network (www.tropnet.net) and ProMED mail (www.promedmail.org).
§Seroprevalence study

(Source: Amarasinghe et al., 2011)
2.7 Impact of Dengue Virus infections

2.7.1 Public Health Impact
The real public health impact of DF/DHF occurs during epidemics of this disease. Due to the similarity of clinical symptoms with other febrile illnesses, the early stages of epidemic transmission are usually not detected, with cases grossly under-reported until the epidemic is recognized as dengue, which is usually near peak transmission; it then becomes grossly over reported. Emergency mosquito control is usually initiated at that time, but these efforts are usually misdirected, and are too little and too late to have any impact on the epidemic. Thus, the public health impact of epidemic DF/DHF is amplified because of no effective preventive measures, no public health planning and no properly implemented emergency response plans (Gubler, 2002).

2.7.2 Social Impact
Dengue afflicts all levels of society but the burden may be higher among the poorest who grow up in communities with inadequate water supply and solid waste infrastructures, and where conditions are most favourable for multiplication of the main vector, *Ae. aegypti* (WHO, 2012).

2.7.3 Economic Impact
Dengue causes a substantial burden to the patients not only physical pain but also economic hardship to them and their family because of health clinic visits, hospitalization, medication, travel expenses, and parents’ time seeking for treatment of their children and disruption of earning potential. The government also has to allocate vast amounts of money for public awareness campaigns, medical services and vector eradication efforts. Another indirect cost comes in the form of loss of revenue through reduced tourism (Gubler, 2002).
2.8 Dengue Virus

Dengue Virus (DENV) is a member of the family *Flaviviridae*, genus *Flavivirus* that consists of 55 identified virus species (ICTVdB, 2006). DENVs are composed of four *flaviviruses* that are closely related but antigenically distinct groups known as serotypes, denoted as dengue virus type 1 (DENV-1), dengue virus type 2 (DENV-2), dengue virus type 3 (DENV-3) and dengue virus type 4 (DENV-4). They have common epitopes on the envelope protein that result in extensive cross-reactions in serologic tests. These make unequivocal serological diagnosis of flavivirus difficult, which is especially true among the four dengue viruses (Zanotto et al., 1996).

The virus is a spherical particle approximately 40-60 nm in diameter consisting of a 30 nm isometric nucleocapsid core surrounded by envelope approximately 10 nm thick. The nucleocapsid is composed of core proteins and houses the viral genomic RNA (Figure 2.9). The genome is a non-segmented, single-stranded, positive-sense RNA and is 10.7 kb in length (Chambers et al., 1990). The DENV open reading frame (ORF) is flanked at its 5’ terminus by an untranslated region (UTR) of about 100 nucleotides and a longer UTR of about 500 nucleotides at its 3’ terminus. The 5’ terminus of the genome has a type I cap (m$^7$GpppAmp) and there is no polyadenylation of the 3’ terminus (Perera and Kuhn, 2008).
The translated polyprotein is cleaved co- and post-translationally by viral and host proteases into ten viral proteins: three structural proteins (C, capsid; prM/M, precursor of membrane/membrane; E, envelope) encoded at the 5’ end of the ORF, and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) encoded at the 3’ end (Figure 2.10). The three structural proteins constitute the DENV virion: the capsid protein surrounds the viral RNA genome to form the nucleocapsid, whereas the prM and E proteins are embedded in the cell-derived lipid bilayer membrane that forms the viral envelope (Perera and Kuhn, 2008)
Figure 2.10 Schematic diagram showing: (top) gene organisation in the dengue virus RNA genome, (bottom) the membrane topology and proteolytic cleavage sites of the transcribed polyprotein. Cellular and viral proteases, which are denoted by arrows, process the immature polyprotein into ten separate proteins (Perera and Kuhn, 2008).

2.9 Pathogenesis Mechanisms of DENV

The mechanisms leading to the severe manifestations of DENV infections are still not completely understood but are likely to be multifactorial. These include: pre-existing DENV immunity and viral genotype. Based on these observations, it has been proposed that viral virulence and aberrant host immune responses are responsible for the development of severe disease (Guzman and Kouri, 2003).

Once the virus is introduced into a human host through the bite of an infected mosquito, released viral particles infect tissue-resident cells via the mannose receptor on macrophages, dendritic cells-specific ICAM-3-grabbing non-integrin 1 (DC-SIGN) on dendritic cells (DC) and activate resident immune cells such as mast cells (Tassaneetrithep et al., 2003; Miller et
Langerhans cells have also been identified to be permissive to DENV infection (Wu et al., 2000). The activated mast cell mediates a local inflammatory response to DENV in the skin that prompts the recruitment of leukocytes from the vasculature, including natural killer (NK) cells and NK T cells, which promote the killing of virus-infected cells at the site of injection (Figure 2.11) (John et al., 2011).

However, the infected DC mature and migrate to local or regional lymph nodes where they present viral antigens to T cells. The infected cells releases virus that infect other cell types in the draining lymphnode such as monocyte/macrophages, B cells, and other DCs. DENV replicates in DC, macrophages, and other monocytes, seeding a viremia. From the draining lymph nodes, the virus enter the bloodstream, possibly via infected B cells, which mediates infection of secondary organs such as liver, kidneys, and spleen (Jessie et al., 2004).

![Figure 2.11 Host responses to cutaneous dengue virus injection (John et al., 2011)](image-url)
Symptomatic infections leading to DF are preceded by an incubation period generally ranging from 4 to 10 days. The acute phase of illness lasts for 3 to 7 days post onset of fever and is generally self-limiting (WHO, 2009). Resolution of DENV infection is associated with virus clearance by cytotoxic T cells and virus neutralization by antibodies that can block virus-mediated cell membrane fusion or virus attachment by targeting DII and DIII of the E protein respectively (Crill and Roehrig, 2001; Yauch et al., 2009).

Around the time of defervescence, DF can progress to DHF and DSS, which are characterized by thrombocytopenia, hemorrhagic manifestations, and increased vascular permeability that can lead to hypovolemic shock (Gubler, 1998; WHO, 2009). The four theories explaining why disease pathogenesis can progress to DHF and DSS include; Antibody Depended Enhancement (ADE), virus virulence, T-cell mediated and molecular mimicry that result in increased virulence.

### Table 2.2 Existing hypotheses to explain DENV pathogenesis

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Mechanism</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Depended Enhancement</td>
<td>Immune-complexes</td>
<td>Enhancement of infection of target cells</td>
</tr>
<tr>
<td>Viral virulence</td>
<td>Highly virulent strains</td>
<td>Increased infection of target cells</td>
</tr>
<tr>
<td>T-cell mediated</td>
<td>Cytokine production</td>
<td>Increased vascular permeability</td>
</tr>
<tr>
<td>Molecular mimicry</td>
<td>Autoimmune reactions</td>
<td>Hemorrhagic manifestations</td>
</tr>
</tbody>
</table>

The ADE hypothesis states that upon secondary infection with a heterologous DENV serotype, pre-existing, sub-neutralizing, and non-protective antibodies will bind to viruses and will enhance their uptake in FcR-bearing monocytic cells, resulting in enhanced infection and greater burden of disease (Figure 2.12) (Whitehead et al., 2007). Concurrent with the antibody response to heterologous infection, activation of memory T-cells specific for the previous infection (original antigenic sin) is postulated to delay viral clearance and increase
cytokine production, thereby skewing the immune response away from the current infection (Mongkolsapaya et al., 2003). However, original antigenic sin is not always associated with ADE disease progression. Infants can develop DHF without experiencing a prior infection, suggesting that ADE associated disease in infants is mediated primarily by maternal antibodies (Halstead et al., 2002). Additionally, infants who have no maternal antibodies between ages 1-3 years also have a potential to develop DHF due to unknown mechanisms aside from ADE (Ngwe Tun et al., 2013).

Figure 2.12 Model for antibody-dependent enhancement (ADE) of dengue virus replication (Whitehead et al., 2007).

The memory CD4+ T cells activated by infection release Interferon gamma, which will upregulate expression of FcR on monocytes, and further perpetuate the infection of these cells (Figure 2.12) (Pang et al., 2007). In response to infection with DENV, macrophages, dendritic cells and other infected mononuclear cells, secrete vasoactive mediators, which cause vascular permeability and may lead to shock, the most important feature of severe DENV infection. It is hypothesized that a complicated network of cytokines and other mediators, involving TNF, interleukin-1 (IL-1), IL-2, IL-6 IL-8, macrophage inflammatory
protein (MIP)-1α, interferon-inducible protein (IP)-10, and platelet activation factor (PAF) as well as complement activation products such as C3a and C5a and histamine may be responsible for increased haemorrhage and vascular permeability (Chaturvedi et al., 2000; Espina et al., 2003).

During DENV infection, viral antigens are presented by infected cells, in the context of MHC antigens resulting in priming and stimulation of CD4+ and CD8+ T cells. A consequence of T cell activation is the production of several cytokines including IL-2, IL-4, IL-5 and IL-6, whereas infected macrophages produce TNF, PAF, IL-1 and IL-6 while monocytes produce TNF-α, IL-1β, IL-6, and IL-8 and anti-inflammatory cytokines IL-10 and TGFβ. This complicated network acts synergistically, resulting in increased vascular permeability observed during DHF and DSS (Chaturvedi et al., 2000; Gerber and Mosser, 2001).

In addition, the production of IFN-α during DENV infection up-regulates the expression of Fc receptors and MHC expression, which results in increased numbers of DENV infected cells. These chain reactions and production of the cytokine cascade results in immunopathology seen in DHF and DSS (Figure 2.13) (Chaturvedi et al., 2000; Gerber and Mosser, 2001). Amongst the up-regulated cytokines in severe dengue cases, TNFα is of particular interest because of its ability to increase endothelial permeability (Yen et al., 2009). Notably, TNFα308A allele, which leads to overproduction of this cytokine, is associated with DHF (Perez et al., 2010). Another cytokine, IL-10 result in reduced levels of nitric oxide and hence increased levels of pro-inflammatory cytokines. IL-10 has also been shown to correlate with platelet decay and reduced function, resulting in enhanced disease severity (Libraty et al., 2002). IL-6 elevates level of tissue plasminogen activator which is important in the control of fibrinolysis (Duchini et al., 1996).
Figure 2.13  **Model of T cell mediated immunopathogenesis in DHF leading to plasma leakage.** According to this model, during secondary infection, memory DENV specific T cells are able to recognize DENV infected cells more rapidly than naive cells, become activated in shorter time and secrete high amounts of the cytokines leading to increased vascular permeability and plasma leakage (Chaturvedi *et al.*, 2000).

Effects from produced by different cytokines suggests that it is likely that multiple cytokines and soluble factors contribute simultaneously in a complex way to result in DHF/DSS. On the other hand, overproduction of matrix metalloproteinase (MMP) 9 by immature DC infected by the dengue virus is involved in the enhanced endothelial permeability (Luplerdlop *et al.*, 2006)

The existences of genotypic variations that make some strains more virulent and are associated with greater severity of illness have been proposed. The analysis of whole genome sequence of DENV-2 causing either DF of DHF revealed that determinants of DHF occur at amino 390 of envelope (E) protein, the downstream loop (nucleotides 68-80) of the 5’ noncoding region and the upstream 300 nucleotides of the 3’ NCR. These changes in the
virus genome implicated risk factors for the development of severe disease (Leitmeyer et al., 1999; Fried et al., 2010; Ty Hang et al., 2010). Disease severity has also been associated with high viremia titers in the infected human and differences in virus output from infected monocytes (Vaughn et al., 2000; Cologna and Rico-Hesse, 2003).

Molecular mimicry resulting in autoimmune reactions has also been an alternative to explain the pathogenesis of DHF and DSS. It has been demonstrated that part of the DENV envelope protein, a 20-amino acid sequence, shares a sequence similarity with a family of clotting factors, including plasminogen and in addition, cross-reactive antibodies to plasminogen appeared during DENV infections (Markoff et al., 1991). These findings indicate that a relation between these cross-reactive antibodies and development of haemorrhagic manifestations may exist. In addition, antibodies to the NS 1 protein have been found to cross-react with epitopes on human blood clotting factors and integrins and bind to endothelial cells (Falconar, 1997). In addition, formation of platelet-associated immunoglobulins play a critical role in the mechanisms of thrombocytopenia and the accompanying increased vascular permeability. It has been demonstrated that increased platelet activation IgG (PAIgG) levels which involve anti-DENV IgG, were closely associated with thrombocytopenia during the acute phase of secondary infection (Oishi et al., 2003; Saito et al., 2004).

2.10 Laboratory diagnosis of Dengue Virus infection

The current laboratory diagnosis of dengue is based on the detection of markers of DENV infection in patient serum (Peeling et al., 2010). These include the viral components and antibodies that are present in the serum at different time points of the infection (Figure 2.14).
The tests are based on the immunological response after exposure to an external agent such as virus. Immunoglobulin M (IgM) and immunoglobulin G (IgG), singly or in combination, are usually the key factors to be detected in the diagnosis of dengue primary infection and secondary infection (Figure 2.14). Detection of DENV IgM in the low titer of DENV IgG (i.e. an IgM positive and IgG negative reactivity pattern) is a clear indicator of primary DENV infection (De Souza et al. 2007). An IgM positive and high titer IgG reactivity pattern is an accurate marker of secondary infection among patients whose serum samples were collected after 7 days from onset of fever. In DHF and DSS, antibody response has been grouped depending on the disease severity. This includes; high IgM titer with low anti-flavi IgG titer, low IgM titer with high anti-flavi IgG titer, and high IgM titer with high anti-flavi IgG titer (Ngwe Tun et al., 2013).
Immunoglobulin M is a reliable marker in infants for primary immune response since it does not cross react with the flaviviruses. However, positivity of anti-dengue IgG usually implicates the maternal protective antibody transmitted through the placenta during gestation period (Hammond et al., 2005).

Other antibodies such as DENV specific IgA antibodies are also present during acute phase DENV infection; however they disappear early in convalescence, approximately three to four weeks after infection (Nawa et al., 2005).

The following are serologic tests used for the diagnosis of DENV infection.

a) **Haemagglutination Inhibition Test**

The haemagglutination inhibition (HI) test has been the most frequently used test for routine serologic diagnosis of dengue. It is sensitive, easy to perform, requires only minimal equipment, and is very reliable if properly done. The HI test is based on the fact that the DENV, under controlled conditions of pH and temperature, can agglutinate goose red blood cells, and this effect can be inhibited by specific antibodies (Figure 2.15). The antigens employed are prepared from infected suckling mice brains by extraction with acetone to remove the lipids, or from infected mosquito cell cultures fluid that have been concentrated. Serum specimens must be treated at 56°C for 30 minutes to remove non-specific inhibitors, followed by adsorption to 20% goose erythrocytes to observe natural hemagglutination. The major disadvantage of the HI test is lack of specificity, which makes the test unreliable for identifying the infecting virus serotype. However, some primary infections may show a relatively monotypic HI response that generally correlates with the virus isolated (WHO, 2009; Inoue et al., 2010).
b) Neutralization Test

The neutralization test (NT) is the most specific serologic test for dengue viruses. The most common protocol used in most dengue laboratories is the serum dilution plaque reduction neutralization test (PRNT). It is based on the fact that dengue viruses produce cytopathic effects (CPE) which can be observed as plaques in susceptible cell cultures. This CPE is neutralized by the presence of specific antibodies. Since NT is more specific than HI, it can be used to identify the infecting virus in primary dengue infections, provided the serum samples are properly timed. Relatively monotypic responses are observed in properly timed convalescent-phase serum. In secondary and tertiary infections, it is not possible to reliably determine the infecting virus serotype by NT. The major disadvantages are time required to perform the test, and technical difficulty (WHO, 2009).

c) IgM-Capture Enzyme Linked Immunosorbent Assay

IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA) is widely used. It is based on detecting the dengue-specific IgM in the test serum by capturing them out of solution using anti-human IgM that was previously bound to the solid phase. If the IgM from the patient’s serum is anti-dengue antibody, it will bind the dengue antigen that is added in the next step and can be detected by subsequent addition of a horseradish peroxidase labeled anti-
dengue antibody, which may be monoclonal antibody. An enzyme o-phenylenediamine complex substrate is added to give a colour reaction (Figure 2.16). MAC-ELISA is a valuable tool for the dengue diagnosis and surveillance. During epidemics, MAC-ELISA has the advantage of fast detection in propagation of the transmission. In areas where dengue is endemic, MAC-ELISA can be used as a valuable tool in the evaluation of a great number of clinical samples, with relative ease (WHO, 2009).

Figure 2.16 IgM-capture ELISA

**d) Indirect IgG-ELISA**

An indirect IgG-ELISA has been developed and has higher sensitivity than the HI test. It can be used for the differentiation of primary and secondary infections by dengue (Figure 2.17). The test is simple and easy to do, and it can be used in the analysis of a large number of samples (WHO, 2009). The indirect IgG-ELISA exhibits the same broad cross-reactivity among flaviviruses. Therefore, it cannot be used to identify the infecting dengue serotype.
e) **Non-structural protein 1 (NS1) ELISA**

NS1 of the dengue viral protein has been shown to be useful as a tool for the diagnosis of acute dengue infections. Although NS 1 protein is not structural protein to form virus, it is well known to be secreted from infected cells. Thus, DENV NS1 antigen has been detected in the serum of DENV infected patients as early as 1-day post onset of fever, and up to 18 days post onset of fever. The NS1 ELISA based antigen assay has been introduced recently for DENV detection and many investigators are evaluating its sensitivity and specificity. The NS1 assay is useful for differential diagnostics between flaviviruses because of its specificity (CDC, 2012b)

f) **Rapid diagnostic tests (RDT)**

A number of RDT kits for anti-dengue IgM and IgG antibodies are commercially available (WHO, 1997).

### 2.11 Dengue Treatment

It is recommended that patients with DF have rest. Doctors can treat symptoms of DF with antipyretics and anti-emetics (WHO, 2009). Aspirin (Acetylsalicylic acid) is not recommended in dengue patients due to its anticoagulation effects and the risk of Reyes
Syndrome in children. Hospitalization and admission to the intensive care unit is recommended if patients present with signs of severe hemo-concentration or shock (WHO, 2009). The mainstay of DHF/DSS treatment is intravenous fluid in the form of isotonic solutions or Ringers for twenty-four hours or until the patient’s hematocrit drops (WHO, 2009). Patients may also be transfused if they lose excessive amounts of blood due to hemorrhage (WHO, 2009). Hospitalization is recommended for at least twenty-four hours following defervescence and patients should not be discharged until they meet the following criteria: return of appetite, clinical improvement, no respiratory distress, stable hematocrit, platelets greater than 50,000/mm$^3$, and good urine output (WHO, 2009). DSS patients should remain hospitalized for at least two days following their recovery from shock (WHO, 2009).

2.12 Dengue Vaccine and Drug Development

There are difficulties in the production of dengue vaccine because of the concerns about antibody dependent enhancement and increased severity of secondary infections (Stephenson, 2005). Immune response to heterologous dengue antigens has potential to tilt the balance from protection to immunopathology (Huisman et al., 2009; Rothman, 2004; Durbin and Whitehead, 2010). The lack of an appropriate animal model and the poor understanding of the pathogenesis of dengue have made the development of an effective and safe dengue vaccine difficult (Yauch and Shresta, 2008). An optimal vaccine need to meet many requirements to produce balanced immunity to all four serotypes, to cause minimal vaccine-induced severe type of DENV infection, to produce lifelong protection, and be economically feasible (Whitehead et al., 2007).

Compounds inhibiting DENV replication, enzymatic activity, receptor binding and fusion are currently under investigation (Morrison et al., 2010). The aim of these studies is to identify potential drugs that can prevent development of DHF and DSS from DF (Noble et al., 2010).
Additionally, therapeutic antibodies are currently being studied for their potential in treatment of dengue (Shrestha et al., 2010). The current approaches of dengue vaccine development include chimeric, live attenuated, inactivated, sub-unit vaccine and DNA vaccine. Sanofi Pasteur’s ChimeriVax-DENV vaccine has recently entered phase 3 clinical testing (Coller and Clements, 2011). This chimeric vaccine consists of structural genes (prM and E) from four DENV serotypes inserted into the yellow fever virus 17D vaccine strain as a backbone (Figure 2.19) (Guy et al., 2010). Results from phase 1 and 2 have been positive as the vaccine was shown to be safe and immunogenic, with cell-mediated immunity biasing towards interferon-gamma (IFNγ) rather than tumour necrosis factor-alpha (TNFα) (Guy et al., 2010).

Presently, two leading live-attenuated tetravalent formulations have been tested in phase 1 and 2 clinical testing but these vaccines have been put on hold as the vaccinees experienced adverse reactions and imbalanced serological response towards the four DENV serotypes (Kitchener et al., 2006, Sanchez et al., 2006), possibly attributed to interference between dengue serotypes in tetravalent formulations (Anderson et al., 2011). Inviragen Inc, in collaboration with the Centers for Disease Control and Prevention, USA, tapped on the DENV-2 (16681-PDK53) strain that was derived through serial passage in primary dog kidney cells as the genomic backbone. prM and E genes of DENV1, 3 and 4 replaced the DENV 2 genes to form chimeras that make up the rest of the tetravelant formulation (Figure 2.18). This vaccine, named DENVax, has also entered phase 2 clinical trials (Osorio et al., 2011). Other inactivated vaccines have also been considered, such as psoralen-inactivated vaccines which have been shown to be immunogenic and can reduce viraemia after experimental challenge in vivo (Maves et al., 2011).
The Sanofi Pasteur vaccine uses a yellow fever virus backbone with prM and E segments from the other four DENV serotypes. The NIH vaccine is a chimeric dengue vaccine that uses DENV-4 (with 30 nucleotides deleted at the 3’ end of the genome) as the backbone. The CDC Inviragen vaccine is a mixture of four recombinant DENV-2 genomes. In contrast, the Merck/Hawaii Biotech is a subunit vaccine containing E proteins from four DENV serotypes. Another type of vaccine under clinical development is DNA vaccine (Rothman, 2011).

DNA vaccines developed by the US Naval Medical Research Center have completed a phase-1 trial testing a DENV-1 prM/E DNA vaccine (Thomas SJ, 2011). To improve immunogenicity and protection, different vector and adjuvant combinations have been considered. For example, DNA vaccines comprising of prM-E genes in a Venezuelan equine encephalitis virus replicon particle system is under clinical development (Chen et al., 2007). The use of better adjuvants, such as Vaxfectin®, has been shown to improve DNA vaccines by increasing their immunogenicity and protection, highlighting its potential to improve tetravalent dengue DNA vaccines (Porter et al., 2012).
2.13 Methods of Vector Control

The most effective way for preventing DENV transmission depends entirely on control of the mosquito vectors (Aedes mosquitoes) or interruption of human–vector contact. Activities to control transmission should target *Ae. aegypti* in habitats of its immature and adult stages in the household and immediate vicinity, as well as other settings where human vector contact occurs (Henchal and Putnak, 1990).

2.13.1 Environmental Management

Environment management seeks to change the environment in order to prevent or minimize vector propagation and human contact with the vector-pathogen by destroying, altering, removing, or recycling non-essential containers that provide larval habitats. The choice of approach should be effective, practicable, and appropriate to local circumstances (WHO, 2009).

2.13.2 Chemical Control

Current methods for applying insecticides include larvicide application, perifocal treatment, and space spraying. Three larvicides have been used to treat water containers; 1% temephos sand granules, insect growth regulator methoprene in form of briquettes, and BTI (*baccilus thurungiensis* H-14), which is considered below in the section on biological control. Perifocal treatment involves the use of hand or power sprayers to apply wettable power or emulsifiable concentrated formulations of insecticide as spray to larval habitats and peripheral arrears. The insecticides currently in the perifocal treatment are; Malathion, Fenitrothion, Fenthion and some Pyrethroids. Space spraying is the spreading of microscopic droplets of insecticides in the air to kill adult mosquitoes and is used in emergency situations when an outbreak of dengue fever is already in progress. Two forms of spray are generally used, ultra-low volume aerosols (cold fogs and mist). However, Insecticide resistance must be considered as a
potentially serious threat to effective dengue vector control. Routine monitoring of insecticide susceptibility should be an integral part of any control programme (WHO, 2009).

2.13.3 Personal Protection
Pyrethroid-impregnated bed nets or curtains are effective against night feeding mosquitoes and are useful for bed-ridden, infants or day sleep persons. Commercially available insect repellents can be used for tourists and short-term visitors to dengue endemic areas. For residents and those staying longer in endemic areas, clothing can be impregnated with Permethrin (WHO, 2009).

2.13.4 Biological Control
Larvivorous fish and the biocide Bacillus thruringiensis H-14 (BTI) are the two organisms most frequently employed (WHO, 2009).

2.13.5 Integrated Control
Integrated vector control is the combination of available control methods in the most effective, economical and safe manner to maintain vector population at acceptable levels. Environmental management of DENV vectors can be successfully combined with health education and public awareness, where source reduction activities are promoted by local health care workers (WHO, 2009).
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Site

This study was conducted at the Coast Provincial General Hospital (CPGH) that provides the health care services to the local people and serves as a referral center to the entire County. The facility provides a variety of health care services through inpatient and outpatient departments under the units of medicine, surgery, gynecology, and other medical subspecialties (e.g. pediatrics, obstetrics, and microbiology). CPGH facility is located in the County of Mombasa, found on Kenya’s eastern coastline bordering Indian Ocean. The County is one of Africa’s major tourist destinations, with some of the best beaches in the world. It has an international airport and a prominent busy seaport linking east and central Africa to rest of the world. The County covers an area of 2,755km² and lies between latitudes 3°56’ and 4°10’ south of equator and longitudes 39°34’ and 39°46’ east. It is characterized by a distinctive hot and humid climate influenced by South East and North East monsoons. This area receives heavy downpours between April and May and the occasional showers towards the end of the year. The rainfall ranges from moderate to high, with an average of 1,162 mm annually. The average temperature is 26.5 °C (20 °C – 33 °C). Mean relative humidity for an average year is recorded as 77.6%.

The total population size is 939,370 as per the 2009 census (Kenya National Bureau of Statistics, 2009) with human population density of 4,292 per km² and 37.6% of this population live below the poverty line (Kenya Integrated House Budget Survey, 2007). The literacy levels may be up to 86.2%, being higher in urban than rural areas. The increase in human population density and widespread poverty at the coastal region of Kenya, contribute to conditions that modify the environment and directly influence the risk of DENV transmission. These conditions include; poor physical planning that result into haphazard
building, inadequate sewage and waste management systems, and deficiencies in water supply systems leading to water storage practices. Furthermore, poor garbage control, mass production of non-biodegradable plastic containers and used automobile tires discarded in environment provide important sites for mosquito breeding.

However, Mombasa County attracts a large number of visitors/tourists, and migrants most of whom are employed in the commercial port and the industrial zones of the County, thus increasing the vulnerability of the county for DENV. Lastly, globalization also results to more international contact and travel through the exchange in population and goods which increases the risk of imported dengue in County, thus increasing the domestic spread of the disease.

3.2 Study Design

This was a hospital-based prospective study conducted for a period of 6 months (February to July 2012).

3.3 Variables

The variable in the present study included age, gender, and month as independent variables, while dengue patient as dependent variable in this study.

3.4 Study Population

This study was performed among febrile patients seeking medical care at both the inpatient and outpatient departments. The patients were selected according to the following criteria:

a) Case inclusion criteria:

The study included patients aged above 2 months with symptoms of fever (38.5–41.4°C) and with more than or equal to two of the following: 1) Joint pain, 2) Rash, 3) Myalgia, 4) Headache, 5) Retro-ocular pain 6) Abdominal pain and 6) Hemorrhagic manifestation
(WHO, 2009). Before recruitment in the study, the patient, parent, or guardian provided a written informed consent.

**b) Case exclusion criteria**

The study excluded children less than 2 months old, patients with fevers of known cause, and those patients who were unable or unwilling to give a written consent.

### 3.5 Sample Size

It was assumed that the patients attending CPGH during the study period were a representation of the total Mombasa County population. The sample size was determined using the Cochran formula with estimated prevalence of 50% (Bartlett *et al.*, 2001; Cochran, 1977)

\[
N = \frac{Z^2 P (1-P) D}{d^2}
\]

Where; 
- **N** - Minimum number of sample required
- **Z** - Standard normal distribution at 5% significance level (1.96)
- **P** - Estimated prevalence of Dengue fever for the coastal region (50%, 0.5)
- **D** - Design effect (1)
- **d** - Degree of precision (5%, 0.05)

\[
N = \frac{1.96^2 (0.5)(1-0.5)1}{0.05^2}
\]

\[
N = 384
\]

Therefore, the minimum blood samples required for the study was 384 = 390 samples. Since, there were anomalies in patients’ response.
3.6 Study Procedure

3.6.1 Recruitment of Patients
A trained study clinical officer recruited eligible patients and collected data at pediatric, outpatient and inpatient departments of CPGH. The study clinical officer introduced himself and explained to the parents and guardians the purpose of the study. Informed verbal and written consent was obtained from parents and guardians who allowed their children to take part in the study (Appendix A). The patients with the guardian were assured of confidentiality of the information. Participation in the study was on a voluntary basis.

3.6.2 Clinical and Demographic Data Collection
A structured assessment form was used to obtain the clinical history regarding febrile illness including clinical symptoms and signs (Appendix B).

3.6.3 Blood Sample Collection Procedure
The study clinical officer collected venous blood samples aseptically from the study participants as follows: The veins in the antecubital fossa or dorsum of the hand were identified and a tourniquet applied to make the veins visible. The area was then cleansed with an alcohol swab and allowed to air dry, 3-5ml of blood was drawn from each febrile patient using a sterile needle and syringe or vacutainer needle and serum separating tube (SST) (Becton Dickinson, SA).

3.6.4 Sample Handling, Transport and Storage
The blood samples were centrifuged at 1,300 x g for 10 minutes at 4°C. A sterile, graduated, disposable transfer pipette was used to transfer serum into two sterile screw-capped cryotubes (1.5 ml per tube, Greiner Bio-One, Germany) and stored at -80°C until testing. The serum samples were collected and delivered to the Kenya Medical Research Institute, Production Department (KEMRI-PD) laboratories, Nairobi.
3.7 Laboratory Procedures

3.7.1 Cell Lines and Virus Strains

* Aeles albopictus* mosquito derived C6/36 cells and African green monkey kidney derived Vero cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% v/v heat inactivated fetal bovine serum (FBS Sigma, USA) and 100 units/ml penicillin, 100µg/ml streptomycin and 292 µg/ml L-glutamine (GIBCO), 0.1% non-essential amino acids (Gibco/Invitrogen, UK) and 2-3% Sodium bi-carbonate. C6/36 and Vero cells were cultured in 25 cm², 75 cm² tissue culture flasks (Nunc, Denmark) at 28°C and 37°C, respectively. The cell lines were passaged every 5-7 days. The cell monolayer was washed with 0.1% trypsin in 0.02% EDTA solution was added for 3 minutes at 28°C and 37°C, respectively. After addition of trypsin-EDTA solution, the flask was tapped to detach and disperse cells. Equal volume of culture medium was added to stop the enzyme activity and cell suspension centrifuged at 1,400 rpm for 4 minutes. The cell precipitate was re-suspended with growth medium and transferred into flasks.

The DENV strains used in this study were: DENV-1 (Hawaii), DENV-2 (00St-22A), DENV-3 (SLMC-50), and DENV-4 (SLMC-318). All the strains were grown in the C6/36 cells at 28°C for 7-10 days and stored in aliquots at -80°C as seed virus stock until use.

3.7.2 Antigen Production

3.7.2.1 Propagation and Harvesting of the Virus

*Aedes albopictus* clone C6/36 cell line was grown at 28°C in MEM with 10% FBS in Roux bottles. At 80% confluence, growth medium was removed and 1 ml of seed virus inoculated in each bottle, followed by 2 hours virus adsorption at 28°C. The inoculum was spread over the cell sheet every 20 minutes. Thereafter, maintenance medium was added to cell sheet and incubated at 28°C. After 14 days for DENV-1, 9 days for DENV-2, 12 days for DENV-3, and
10 days for DENV-4, the infected culture fluids (ICF) were collected in centrifuge bottles (Beckman Instruments, USA) and spun at 5000 rpm for 10 minutes at 4°C in a JLA-10.500 rotor (Beckman Instruments, USA) in Avanti J-26 XP centrifuge to remove cell debris.

### 3.7.2.2 Virus Concentration Using Jumbosep™ Centrifugal Devices

#### a) Principle of the procedure

Centrifugation up to 3,000 x g provides the driving force for filtration, moving sample toward the highly selective, low protein-binding Omega™ membrane. Molecules larger than the membrane’s nominal molecular weight cutoff of 30K (MWCO-30K) are retained in the sample reservoir. Solutes and molecules smaller than the MWCO-30K of the membrane pass through the membrane surface into the membrane insert and through the filtrate port into the filtrate receiver (Pall Life Sciences, 2007).

![Jumbosep device components](image)

**Figure 3.1 Jumbosep device components** (Pall Life Sciences, 2007).

#### b) Procedure

The procedure was performed by following manufacturer’s instruction. The filtrate receiver was separate from the sample reservoir and membrane insert with the filtrate port facing down dropped into the sample reservoir (Figure 3.1). The sample reservoir was placed on a
hard surface and membrane insert pressed down firmly to rest on the knobs at the bottom of the sample reservoir. Empty filtrate receiver was attached to the bottom of the sample reservoir, 60 ml of ICF was added to the sample reservoir and capped to prevent evaporation during centrifugation. The Jumbosep devices were placed in a swinging-bucket rotor (B438-29) that accepted standard 250 ml bottles and spun at 4,200rpm for 60 minutes at 4 °C in Tomy AX-311 versatile refrigerated centrifuge (Tomy, Japan). Jumbosep devices were removed at the end of spun time and sample reservoir separated from the filtrate receiver. Retentate was recovered by pouring off the retentate into pre-labeled 15 ml centrifuge tubes, a pipette tip sledded under the dislodged membrane insert and remaining retentate removed. The retentate fluid was then stored at -80 °C.

3.7.3 Sandwich ELISA to Assay Dengue Antigen Titer

The principle of Voller et al., (1976) was used with some modifications (Bundo and Igarashi, 1985) A 96-well ELISA flat bottom plate was coated with anti-flavivirus IgG (20µg/ml) in coating buffer (0.05 M carbonate–bicarbonate buffer, pH 9.6, containing 0.02% sodium azide) at 4 °C overnight. The plate wells were blocked with Blockace (Yukijirushi, Japan) at room temperature (r.t). After washing with PBS-Tween 3 times, test samples, standard antigen, and negative control (MEM) were distributed in duplicate. The plate was incubated at 37 °C and washed as above, and horseradish peroxide (HRPO)-conjugated anti-flavivirus IgG original (1:500 dilution in PBS-Tween) was distributed into all wells except blanks. Unbound conjugate was washed off as above, and the plate was incubated with substrate solution containing α-phenylenediamine dihydrochloride (OPD) and 0.05% hydrogen peroxide for 30 minutes at room temperature in the dark. The reaction was stopped by adding 1N sulfuric acid and optical density (OD) read at 492nm using Multiskan EX ELISA Reader (Thermo Scientific, China).
3.7.4 Preparation of Dengue Tetravalent Antigen

The DENV tetravalent antigen for IgM capture ELISA was prepared by mixing equal titer of DENV 1, 2, 3 and 4 ICF to make 100 ELISA units. The mixture was aliquated in 10ml and stored at -80°C.

3.7.5 Dengue IgM-capture ELISA

An in-house DENV IgM-capture ELISA (in-house IgM ELISA) was carried out following the protocol described by Bundo and Igarashi, (1985). The 96-well flat-bottomed microplate (Maxisorp Nunc, Denmark) was coated with anti-human IgM (µ-chain specific) 5.5 µL/100 µL/well (Cappel, Germany) and diluted with ELISA coating buffer in all wells except blanks. The plate was incubated at 37 °C for 1 h or at 4 °C overnight. All wells except the blank were blocked with 100 µl of the original concentration of Blockace, and were incubated at room temperature (r.t) for 1 h. The reagents were removed from all of the wells by washing three times with phosphate buffered saline containing 0.05% Tween 20 (PBS-T). The test serum samples as well as positive and negative control sera at 1:100 dilutions in PBS-Tween were distributed in duplicate wells and incubated at 37°C for 60 minutes. After the reaction and washing, the DEN tetravalent antigen was distributed into the wells. The plate was incubated at 37 °C for 1h and washed as above. HRPO-conjugated anti-flavivirus IgG monoclonal antibody 12D11/7E8 (1:500 dilution in PBS-T and 10% Blockace) was added into all wells except blanks. After the incubation at 37 °C for 1h, the unbound conjugate was washed off and substrate solution containing OPD and 0.03% hydrogen peroxide was added to all wells to proceed in the dark at r.t. The reaction was stopped by adding 1N sulfuric acid and OD read at 492nm by ELISA plate reader. The ratio of the absorbance of the positive serum and negative serum (P/N) was calculated by dividing OD of serum sample by the OD of the negative control serum. The P/N ratio above or equal to 2.0 was considered positive.
3.7.6 Flavivirus Indirect IgG ELISA
An in-house flavivirus IgG indirect ELISA modified by Inoue et al., (2010) was used in detecting flav IgG to determine primary and secondary dengue virus infections. In this modified procedure, purified Japanese encephalitis virus (JEV) antigen (strain: ML-17) was applied as an assay antigen (Bundo et al., 1986). A 96-well microplate (Nunc International) was coated with 250ng/100µl per well of virus antigen at 4 °C overnight. The wells were blocked with 100µl/well of Blockace at r.t for 1h, washed three times with PBS-T for 3 min each. Test sera were diluted at 1:1000 and standard serum was diluted by two serial from 1:100 up to $2^{12}$ with PBS-T with 10% Blockace were each placed in duplicate wells and incubated at 37°C for 1h. The plate wells were washed as above, and then reacted with 100µl/well of 1: 2000 diluted HRPO-conjugated anti-human IgG goat serum (American Qualex, CA) in PBS-T with 10% Blockace. After 1h incubation at 37 °C, the plates were washed as above and 100µl/well of substrate solution was added in each well. The substrate solution used was described in section 3.7.5. After 30 minutes incubation at r.t in the dark, the reaction was terminated by adding 100µl/well of 1 N sulphuric acid to each well. The OD was read at 492nm by ELISA plate Reader. The IgG titers of patient sera were determined from a positive standard curve. A sample titer ≥ 1:52,000 was considered to be a DENV secondary infection, whereas a sample titer < 1:52,000 was considered to be a DENV primary infection (Inoue et al., 2010).

3.9 Serological Definitions of DENV Infection
a) **Laboratory-positive DENV infection case**: a single positive anti-dengue IgM with P/N ratio equal to or greater than 2.0 according to the WHO case definition (Bundo and Igarashi, 1985; WHO, 2009).

b) **Primary DENV infection case**: A laboratory-positive case in which the IgG-ELISA titer was <1:52,000 (Inoue et al., 2010).
c) **Secondary DENV infection case**: A laboratory-positive case in which the IgG-ELISA titer was ≥1:52,000 (Inoue *et al*., 2010).

### 3.10 Data Storage and Analysis

The data collected and generated in the laboratory was entered in excel spreadsheets in a password protected computer. The data was then converted to Statistical Package for Social Science (SPSS) version 16.0 (SPSS Inc., Chicago, USA) for analysis. The data for the IgG titers from the in-house IgG ELISA were expressed as the geometric mean. An analysis of variance (ANOVA) was used to compare geometric mean of the DENV cases across the age groups and months. A p-value less or equal to 0.05 (p ≤ 0.05) was considered as statistically significant. Microsoft Excel was used to generate all graphs and tables. The relationship of less than or equal to 5% between gender and dengue cases was analyzed using of Fishers exact tests between two categorical variables.

### 3.11 Ethical Consideration

The protocol of the study for data collection was reviewed and approved by Kenyatta University Ethical Review Committee and KEMRI Scientific Steering Committee (Appendix C). The study was described in more detail to the participants and a written informed consent (Appendix A) obtained from participants ≥18 years, and from parents/guardians of participants younger than 18 years. In addition, assent was obtained from participants 6–17 years of age. If participants were unable to read and sign the consent form, oral consent was obtained and documented in the presence of a witness. The consent form provided a description of the study, participant’s name, date of birth, sex, and name of parent or guardian, details on risks associated with venipuncture, the name, and address of investigator. Each participant was assigned with a unique study number to be used for all laboratory and data analyses to ensure patient confidentiality.
CHAPTER FOUR: RESULTS

4.1 Prevalence of Dengue Infection Cases among Febrile Patients

During the study period (Feb – July 2012), a total of 390 serum samples from febrile patients were tested for dengue antibodies using an in-house IgM-capture ELISA and indirect IgG ELISA. The patients were diagnosed for primary DENV infection, secondary DENV infection and non-dengue infection depending on antibody titer against DENV. Fifty four (13.9%) cases were confirmed as dengue infection while 336 (86.1%) cases were found to be non-dengue (Table 4.1).

4.2 Distribution of Dengue Positive Cases by Age

The age of all patients ranged from 2 month to 82 years. The mean age was 24.9 years, with median age of 25 years and standard deviation of 17.2 years. The age was grouped to capture the most vulnerable age group, as it is known that undifferentiated febrile illnesses is more often common among the pre-school children (1-5 years) and infants (< 1 year), therefore may experience more severe clinical outcome after primary dengue infection. (Guzman et al., 2002; Hammond et al., 2005). The highest affected group in the present study were patients aged between 36 - 45 years with 11 (20.4%) and least being children aged 6 - 15 year with 6 (8.3%). There was a significant difference in occurrence of DENV infection by age groups (p = 0.021) as shown in Table 4.1.
Table 4.1 Prevalence of Dengue Virus Infection among Febrile Patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Febrile cases n (%)</th>
<th>Dengue cases (IgM +ve)</th>
<th>Non-dengue patients (IgM -ve) n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Primary infection IgG titer (&lt;1:52,000) n (%)</td>
<td>Secondary infection IgG titer (≥1:52,000) n (%)</td>
</tr>
<tr>
<td>Male</td>
<td>204</td>
<td>19 (9.3)</td>
<td>9 (4.4)</td>
</tr>
<tr>
<td>Female</td>
<td>186</td>
<td>18 (9.7)</td>
<td>8 (4.3)</td>
</tr>
<tr>
<td>Total</td>
<td>390 (100)</td>
<td>37 (9.5)</td>
<td>17 (4.4)</td>
</tr>
</tbody>
</table>

Age (years)

<table>
<thead>
<tr>
<th></th>
<th>n (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>11</td>
<td>1 (9.1)</td>
<td>0 (0.0)</td>
<td>1 (9.1)</td>
<td>10 (90.9)</td>
</tr>
<tr>
<td>1 – 5</td>
<td>49</td>
<td>3 (6.1)</td>
<td>3 (6.1)</td>
<td>6 (12.2)</td>
<td>43 (87.8)</td>
</tr>
<tr>
<td>6 – 15</td>
<td>72</td>
<td>5 (6.9)</td>
<td>1 (1.4)</td>
<td>6 (8.3)</td>
<td>66 (91.7)</td>
</tr>
<tr>
<td>16 - 25</td>
<td>66</td>
<td>6 (9.1)</td>
<td>2 (3.0)</td>
<td>8 (12.1)</td>
<td>58 (87.9)</td>
</tr>
<tr>
<td>26 - 35</td>
<td>90</td>
<td>9 (10.0)</td>
<td>7 (7.8)</td>
<td>16 (17.8)</td>
<td>74 (82.2)</td>
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<tr>
<td>36 - 45</td>
<td>54</td>
<td>8 (14.8)</td>
<td>3 (5.5)</td>
<td>11 (20.4)</td>
<td>43 (79.6)</td>
</tr>
<tr>
<td>≥ 46</td>
<td>48</td>
<td>5 (10.4)</td>
<td>1 (2.1)</td>
<td>6 (12.5)</td>
<td>42 (87.5)</td>
</tr>
<tr>
<td>Total</td>
<td>390(100)</td>
<td>37 (9.5)</td>
<td>17 (4.4)</td>
<td>54 (13.9)</td>
<td>336 (86.1)</td>
</tr>
</tbody>
</table>

Primary DENV infection was mainly observed among patients aged between 36-45 years with 8 (14.8%) and least in patients aged between 1-5 years with 3 (6.1%) (Table 4.1). The difference between primary DENV infection by age groups was statistically significant (p = 0.049).

The highest secondary DENV infection was observed among patients aged between 26-35 years with 7 (7.8%) and infants (< 1 year) were the least affected 0 (0.0%) (Table 4.1). There was significance difference between secondary DENV infection by age groups (p = 0.027)
4.3 Proportion of Primary verses Secondary Dengue Cases

The highest primary DENV infection was observed among patients aged less than 1 year (100.0%) and the lowest among age group 1-5 years (50.0%) (Figure 4.2). Secondary DENV infection was highest in 1-5 years age group (50.0%), followed by 26-35 years age group (43.8%). There was no significant correlation between primary and secondary DENV infection ($p = 0.057$).

![Figure 4.1 Primary verses secondary DENV infection](image)

4.4 Correlation of IgM with IgG Titer among Dengue Positive Patients

Analysis of correlations between IgM and IgG titers for 54 dengue positive cases was divided into four groups depending in the dengue IgM and IgG titer Group A comprised of 1 case of high IgM titer (P/N ratio $\geq 20$) and low anti-flavi IgG titer ($<1:52,000$), group B with 36 cases of low IgM titer and low anti-flavi IgG titer, and group C with 17 cases of low IgM titer
(2 < P/N ratio < 20) and high anti-flavi IgG titer (≥1:52,000) and group D with no case of high IgM titer and high anti-flavi IgG titer (Figure 4.2).

Thirty seven cases found in group A and B were classified as primary DENV infections due to low anti-dengue IgG titers with both high and low anti-dengue IgM levels. The remaining 17 cases in group C were classified as secondary DENV infections due to high anti-dengue IgG titers. There was no correlation between anti DENV IgM and anti-flavi levels among the 54 DENV patients (y = 4.538 - 0.03lx, R^2 = 0.0001, p = 0.068).

**Figure 4.2 Correlation of the anti-DENV IgM and anti-flavi IgG levels among dengue cases.** The solid line indicates the correlation curve of all 54 cases (y = 4.538 - 0.03lx, R^2 = 0.0001, p = 0.068). The dashed horizontal line indicates the division line between the high and low anti-DENV IgM P/N ratios at 20. The dashed vertical line shows 1:52,000 as the cut-off value for anti-dengue IgG titers between primary and secondary DENV infections.
4.5 Anti-flavivirus IgG responses among febrile patients

Anti-flavivirus IgG titers among 390 febrile patients were distributed among the age groups and plotted individually as shown in Table 4.3 and Figure 4.3 A. The highest geometric mean titer (GMT) was observed in the age group of 26-35 years and the lowest in the age group of 1-5 years. The difference of GMT between two age groups were statistically significant ($p = 0.035$) (Table 4.3 and Figure 4.3 A). However, the difference of GMTs between age groups 6-15 years and 26-35 years was not statistically significant ($p = 0.105$).

| Table 4.2 Geometric mean titer (GMT) of anti-flavivirus IgG among febrile patients |
|--------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
|                                      | Age group (years)                   | Total                              |
|                                      | <1  | 1 – 5 | 6 – 15 | 16 – 25 | 26 – 35 | 36 – 45 | ≥46       |
| Febrile cases                        | n   |        |        |        |        |        | 390       |
|                                      | 11  | 49    | 72     | 66     | 90     | 54     | 48        |
| GMT                                  | 18,876 | 11,870* | 13,011 | 17,147 | 20,234* | 17,882 | 15,702 | 16,129 |
| Dengue cases                         | n   |        |        |        |        |        | 54        |
|                                      | 1   | 5     | 6      | 9      | 16     | 11     | 6         |
| GMT                                  | 31,000 | 28,647 | 14,959** | 28,512 | 31,104** | 22,570 | 31,561** | 26,164 |
| Non-dengue cases                     | n   |        |        |        |        |        | 336       |
|                                      | 10  | 44    | 66     | 57     | 74     | 43     | 42        |
| GMT                                  | 15,449 | 10,739* | 12,847* | 15,824 | 18,437* | 16,848 | 14,211 | 14,708 |

n - number of cases

* Geometric mean titer showed statistical significant

** Geometric mean titer showed no statistical differences

The anti-flavivirus IgG titers were further divided into two categories based on the cut off value as dengue case ($\geq 52,000$) and non-dengue case ($< 52,000$) respectively (Inoue et al., 2010). The anti-flavivirus IgG titers among the 54 DENV cases were distributed and plotted individually depending on the age group. The highest GMT was in the age group of ≥ 46 years and the lowest in the age group 6-15 years as shown in Table 4.3 and Figure 4.3 B. The GMT
between these two age groups did not differ significantly \((p = 0.356)\). In addition, there was no significant difference in GMT between age groups 6-15 years and 26-35 years \((p = 0.464)\).

Among the non DENV cases, the highest GMT was observed among the age group 26-35 years and the lowest in age group 1-5 years (Table 4.3). There was a significant difference in the GMT between these two mentioned age groups \((p = 0.047)\). Similarly, there was significant difference in GMT between age groups 6-15 years and 26-35 years \((p = 0.006)\).

![Figure 4.3 A. Distribution of anti-flavi IgG ELISA titers among 390 febrile patients. Closed diamonds represent individually plotted anti-flavi IgG titer in each age group. The dashed horizontal line indicates the cut-off value of which \(\geq 52,000\) was secondary DENV infection.](image_url)
**Figure 4.3 B. Distribution of anti-flavi IgG ELISA titers among 54 DENV infection cases.** Closed diamond represents individually plotted anti-flavi IgG titer in each age group. The dashed horizontal line indicates the cut-off value of which ≥52,000 was secondary DENV infection.

### 4.5 Distribution of Dengue Positive Cases by Gender

The distribution of 54 dengue positive cases between male and female were 28 (51.9%) and 26 (48.1%), respectively (Table 4.1). The male:female ratio was found to be 1:0.93. The relationship between gender and DENV infection was not statistically significant (p = 0.936). However, significant gender differences were observed in the age group 26-35 (p = 0.010) and ≥ 46 years (p = 0.012), respectively (Figure 4.4).
Out of 37 patients suffering from primary DENV infections, 51.4% were males and 48.6% were females (Table 4.5). The most affected groups were females aged between 26-35 years with 44.4% (p = 0.005) and least cases of DENV infection noted in those above 46 years. Majority of males affected with primary DENV were above 36 years (p = 0.019), with the least prevalence observed in those less than 1 year. Gender differences in primary DENV infection was not statistically significant (p = 0.911).
Table 4.3 Distribution of primary dengue cases by age group and gender

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Gender</th>
<th>Total cases</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>&lt; 1</td>
<td>1 (5.3)</td>
<td>0 (0.0)</td>
<td>1 (2.7)</td>
</tr>
<tr>
<td>1 – 5</td>
<td>2 (10.5)</td>
<td>1 (5.6)</td>
<td>3 (8.1)</td>
</tr>
<tr>
<td>6 – 15</td>
<td>3 (15.8)</td>
<td>2 (11.1)</td>
<td>5 (13.5)</td>
</tr>
<tr>
<td>16 – 25</td>
<td>2 (10.5)</td>
<td>4 (22.2)</td>
<td>6 (16.2)</td>
</tr>
<tr>
<td>26 – 35</td>
<td>1 (5.3)</td>
<td>8 (44.4)</td>
<td>9 (24.6)</td>
</tr>
<tr>
<td>36 – 45</td>
<td>5 (26.3)</td>
<td>3 (16.7)</td>
<td>8 (21.6)</td>
</tr>
<tr>
<td>≥ 46</td>
<td>5 (26.3)</td>
<td>0 (0.0)</td>
<td>5 (13.5)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>19 (100)</strong></td>
<td><strong>18 (100)</strong></td>
<td><strong>37 (100)</strong></td>
</tr>
</tbody>
</table>

Out of 17 patients that suffered from secondary DENV infection, 52.9% were males and 47.1% were females (Table 4.6). Males of age group 26-35 years were most affected at 33.3% and least affected group was aged less than 1 year at 0.0%. However, majority of females affected were aged between 26-35 years (50.0%) with least secondary dengue cases in age group < 1 year and ≥ 46 years (0.0%). Gender differences in secondary infection was not significant by age groups (p = 0.737).
### Table 4.4 Distribution of secondary dengue cases by age and gender

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Gender</th>
<th>Total cases</th>
<th>n</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male n (%)</td>
<td>Female n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>1 – 5</td>
<td>2 (22.2)</td>
<td>1 (12.5)</td>
<td>3</td>
<td>17.6</td>
</tr>
<tr>
<td>6 – 15</td>
<td>1 (11.1)</td>
<td>0 (0.0)</td>
<td>1</td>
<td>5.9</td>
</tr>
<tr>
<td>16 – 25</td>
<td>1 (11.1)</td>
<td>1 (12.5)</td>
<td>2</td>
<td>11.8</td>
</tr>
<tr>
<td>26 – 35</td>
<td>3 (33.3)</td>
<td>4 (50.0)</td>
<td>7</td>
<td>41.2</td>
</tr>
<tr>
<td>36 – 45</td>
<td>1 (11.1)</td>
<td>2 (25.0)</td>
<td>3</td>
<td>17.6</td>
</tr>
<tr>
<td>≥ 46</td>
<td>1 (11.1)</td>
<td>0 (0.0)</td>
<td>1</td>
<td>5.9</td>
</tr>
<tr>
<td><strong>Total cases</strong></td>
<td><strong>9 (100)</strong></td>
<td><strong>8 (100)</strong></td>
<td><strong>17 (100)</strong></td>
<td></td>
</tr>
</tbody>
</table>

#### 4.6 Month-wise Distribution of DENV Infection Cases

The highest number of DENV infection was observed during the month of February (24.1%), and the least in the month of July (5.6%). The association between the month and occurrence of disease was not statistically significant \( p = 0.325 \). The relationship between DENV infection and month was as follows; Feb and July \( p = 0.007 \), Feb and May \( p = 0.007 \), May and June \( p = 0.118 \), Jun and July \( p = 0.118 \). There was no significant correlation between DENV infection cases with environmental temperature \( p = 0.077 \) and rainfall \( p = 0.270 \) as shown in Figure 4.5.
Figure 4.5 Correlation between monthly DENV infection and environmental conditions.
CHAPTER FIVE: DISCUSSION

5.1 Prevalence of Dengue Viral Infection

The present study found a prevalence of dengue viral infections to be 13.9% with 9.5% as primary dengue cases and 4.4% as secondary dengue cases. The present study findings appeared to be higher as compared to study findings from the neighboring country (Tanzania) that reported 4.5% and 9.5% of dengue cases among the febrile patients (Vairo et al., 2012; Hertz et al., 2012). The present findings may be as due to the spatial diffusion of the virus and vector proliferation within the region. Since recent studies have reported dengue outbreaks. In 2010, Comoros, Mayotte and Tanzania reported outbreak of dengue fever caused by DENV-3 (Issack et al., 2010; Sante-plus.org, 2010; Klaassen, 2010; Sissoko et al., 2010). DENV infection has also been reported in Mogadishu, Somalia (WHO, 2011). Additionally, the heavy sea bound commercial traffic between eastern Africa and Indian sub-continent where all four serotypes exist, and increased number of tourists and migrants from other endemic areas exposed the coastal region to vulnerability of imported dengue resulting to domestic spread of the disease (Matlani and Chakravarti, 2011).

5.2 Distribution of Dengue Viral Infections by Age

Dengue affects humans of all age groups worldwide and poses a pediatric public health problem in some parts of the world (Gubler, 1998). During the present study, comparison between the different age groups revealed that adults were infected disproportionately to children. The most susceptible age group for DENV infection was 36-45 years and followed by 26-35 years suggesting that the individuals in these age groups were actively involved in outdoor activities that increased their chances of exposure to the infective DENV vector bite. Similar observations have been reported from South East Asia regions where adults were more affected than children (Tank and Jain, 2012).
Regarding children, a lower DENV infection was observed in age group < 1 year (9.1%) in respect to 1-5 years (12.2%). Since the vector *Ae.aegypti*, is a predominantly day biting outdoor vector, Children < 1 year were at a lower risk of dengue infection as they spend most of their time indoors, completely covered or sleep under bed nets unlike the children aged 1-5 year who were able to play and spend more time outdoors within and around the residential areas. A higher DENV infection was observed among children aged 1-5 years with DENV infection cases reaching a low point in the age group 6-15 years before rising again. Similar findings were observed from southeast India and Caribbean (Akram, 1998; Kumar *et al.* 2013). The present findings may be explained by the fact that children aged 1-5 years spent most of their time either at home or at a nursery or kindergarten of which operates within residential areas or shop-houses. However, formal half-day schooling starts at the age of 6 years, often with afterschool extracurricular activities which lead to reduced exposure to mosquito bites among children aged 6-15 years.

Although, secondary infection was highest in children aged 1-5 years, younger children aged < 1 year were at higher risk of severe dengue infection than children age 1-5 years. This was because of maternal antibody enhancement of disease, as maternal antibodies wanes from protective to enhancing levels (Halstead *et al.*, 2002; Hammond *et al.*, 2005). The present study identified striking association of secondary DENV infection with dengue outbreaks. The peak of secondary DENV infection observed among younger children aged 1-5 years could be related to dengue outbreak that occurred in the neighboring Tanzania and Comoros Island in 2010 (Sante-plus.org, 2010; Klaassen, 2010). Another important finding was the evidence of the second peak among adults aged 26-35 years that suggested a link to the major dengue outbreak which affected the coastal region in 1982 (Johnson *et al.*, 1982).
In relation to the antibody levels and dengue severity, dengue IgM P/N ratios were scattered against IgG titers. IgM and IgG levels in groups A-B were considered as having primary DENV infection and group C with secondary DENV infection. This was in agreement with the description given by WHO (2009). However, IgM level in a 9 year patient in group A was remarkably high. This is known to correlate with clinical severity (Lin et al., 2001). Studies have demonstrated that high anti-dengue IgM was involved in the formation of platelet associated IgM (PAIgM) which was associated independently with the development of DHF, representing a possible predictor of DHF with a high specificity (Saito et al., 2004). However, in contrast high IgM levels in the present study did not correlated to severe infection.

Anti-flavi IgG titers from 390 febrile cases were plotted to their age groups. Studies have shown that anti-flavi IgG correlates with severe DENV infections, DHF and DSS (Saito et al., 2004). In the present study, it was observed that adults within the age of 26-35 years not only had the highest DENV infection but also the highest GMT. The results reflected that most adults in this age group were among previous exposures to DENV that occurred during the outbreak in 1982 before quiescence of the disease (Johnson et al., 1982). Additionally, high GMT observed among children aged less than 1 year was because of maternal antibodies to DENV present at birth due to high prevalence of anti-flavi IgG among adult population.

In the present study, there were no severe cases of DENV infection despite the occurrence of secondary DENV infection. The findings are also in agreement with studies carried out in Nepal, Haiti and Brazil where severe forms were not found among the patients of African origin, despite serologic pre-conditions hypothesized to be precursors for DHF (evidence of secondary DENV infection) (Pun et al., 2011; Rico-Hesse et al., 2006; Cardoso et al., 2011). These authors concluded that this was due to polymorphism of human leukocyte antigen
(HLA) and other host genes (for example, transporter associated with antigen processing (TAP) and human platelet antigen (HPA) in black populations. However, the present study gave the snapshot of the disease spectrum during a non-epidemic period.

5.3 Distribution of Dengue Viral Infections by Gender

Gender differences in DENV infection have been inconsistent worldwide, while some studies reporting a higher prevalence in men, others have shown a higher prevalence in women and others no gender difference (Yew et al., 2009; Anker and Arima, 2011). The present study found that both genders were equally susceptible to the DENV infection. The present study finding was in harmony with studies from Nigeria and Madagascar (Sissoko et al., 2010; Dawurung et al., 2010). An increase of DENV infection cases among women aged 26 - 35 years as compared to male of same age group, reflected exposure differences to infected mosquito bites. Indeed, Ae. aegypti breed and rest in human dwellings and surroundings. Therefore, the present findings showed that females in the county of Mombasa were more likely than men to remain in and around the home, carrying out domestic activities during the day when the mosquitoes are most active. Men were exposed only when they return the home by end of day. However, male predominance in adults above 46 years could be partly explained by accumulation of multitypic immunity among the female of the same age. The present study finding was similar studies from India that suggested that exposure to multiple serotypes over a period resulted in development of multitypic immunity in adults (Sharma et al., 2012; Tomashek et al., 2009).

Among the children, gender distribution was reversed with a considerably higher DENV infection among boys aged 0-15 years than girls. The underlying causes of gender differences were not clear but multiple factors could play a role. Plausible explanations could be a biased parents’ health seeking behaviour towards males, differences in innate susceptibility and
clinical presentations. Healthcare-seeking behavior might have accounted for the gender bias observed with more than two times as many boys as girls affected among children less than 15 years. There has been a growing recognition that biological differences between males and females based on genetic, immunological, and hormonal factors may determine the susceptibility to disease and clinical outcomes, including for DENV infection (WHO, 2007b). Other studies have reported a gender disparity of similar magnitude among children, but the relative contributions of innate susceptibility and healthcare seeking behavior have remained unclear (Goh et al., 1987; Agarwal et al., 1999).

5.4 Distribution of Dengue Viral Infections by Month

A number of mathematical models have been developed to account for the seasonality of the disease and the vector. These have incorporated factors such as rain, temperature, humidity, type of land, mosquito density, bite rate and life span (Bartley et al., 2002; Nakhapakorn and Tripathi, 2005). In the present study, highest peak of DENV infection was observed in the month of February, followed by April and March. The finding was in agreement with studies from Saudi Arabia and Pakistan (Ahmed et al., 2008; Siddiqui et al. 2009). Contrary to the present study, other studies have shown that dengue cases coincided mainly with the post monsoon period of subnormal rainfall. This was because of the relatively high prevalence and distribution of Ae. aegypti larval indices after post monsoon rains (Baba and Talle, 2011; Pun et al., 2011).

Occurrence of DENV infection in present study appeared to be independent of rainfall and temperature, implying that factors for DENV transmission were spatially heterogeneous. Presence of DENV infection cases during dry month of March as seen in present study could probably be reflective of the year-round activity of the mosquito vector. The possible reasons influencing the DENV transmission in coastal region of Kenya could include lack of safe
water supply necessitating the storage of water for domestic use that provided suitable breeding sites for *Ae. aegypti*. Additionally, poor garbage control resulted in accumulation of vast numbers of non-biodegradable plastic containers and used automobile tires within the environment that could collect fresh water during the rainy period thereby favoring vector breeding and maintenance of the vector population throughout the year.

The findings of the present study revealed weak temperature induced DENV transmission variations that may have determined vector efficiency. A higher DENV infection during the dry months as compared to rainy months with a monthly peak in February was in support of reports suggesting that temperature affected the rate of development in different mosquito life stages, as well as dengue viral development (Keating, 2001; Yang *et al*., 2009; CDC, 2010). Similarly, other studies have shown that mosquito survival rates were temperature dependent. Studies in Puerto Rico that showed dengue incidence increase one week after every 1°C increase in a weekly maximum temperature (Johansson *et al*., 2009; Jury, 2008). However, the low DENV infection cases during the month of July in the present study, indicated that low temperatures may have slowed viral development and mosquitoes were unlikely to survive long enough to become infectious (Hales *et al*., 2002).

### 5.5 Limitations of the Study

The present study had several limitations. Sample collection was done for only 6 months and this could have been an unusually high or low period for DENV infection, as DENV infection have annual variations. Additionally, the present study findings were based on laboratory-confirmed cases; it was assumed that the actual contribution of asymptomatic cases to the local spread of dengue was unknown and uncertainty about their potential to infect *Ae. aegypti* mosquitoes while viremic. Therefore, the findings might be the tip of the iceberg in the overall pattern of dengue spread in the coast region of Kenya.
6.1 Summary

Dengue is an important emerging disease of the tropical and sub-tropical regions today. It is a complex disease whose symptoms are difficult to distinguish from other common febrile illnesses and can progress from a mild, non-specific viral disease to irreversible shock and death within a few hours. This makes the differential diagnosis problematic especially in the coastal region, where there is a high incidence of febrile illnesses such as typhoid fever and malaria. The study aimed at determining the prevalence of DENV infection and describe the month-wise trend of the disease. A total of 390 serum samples from febrile patients in a period of 6 months (February - July 2012). Dengue antibodies were tested using an in-house IgM-capture ELISA and indirect IgG ELISA. Fifty-four (13.9%) were found to be dengue cases with 37 (9.5%) as primary dengue and 17 (4.4%) as secondary dengue. Majority dengue infections were observed among 36-45 years. Both genders were equally susceptible to the DENV infection. Predominance among female aged 26 -35 years. Lastly, DENV infection occurred throughout the study period with peak dengue infection cases in February.

6.2 Conclusion

The present study concluded that:-

i) A dengue virus infection was one of the causes of acute undifferentiated fever among febrile patients in the county Mombasa.

ii) Children aged less than 5 years were vulnerable to dengue infection and had a greater risk than adults in developing severe forms of the disease when they acquire a second dengue virus infection with a different serotype.

iii) Female predominance in dengue cases among age group 26-25 years would have been masked when collapsing the data over all age groups. Therefore, the present
study findings indicated the importance of reporting age and gender stratified data for dengue surveillance to help in targeting specific preventive measures.

iv) Occurrence of dengue infection during dry month of March reflected a year-round activity of the mosquito vector.

6.3 Recommendations

The present study recommends that:-

i) The government should provide resources at hospital laboratories in County to facilitate early diagnosis and management of dengue patients.

ii) All patients presenting with febrile illness should be tested for dengue antibodies.

iii) Clinicians/physicians consider the possibility of dengue cases when examining febrile patients.

iv) The government should initiate dengue surveillance and commence an integrated vector control programme.

6.4 Further Research

The present study suggested the need to conduct RT-PCR or other antigen based assay in samples collected within short clinical duration (< 6 days) and IgM in those with longer duration, to identify the maximum number of dengue cases. Additionally, research on the circulating serotypes and their genotypes to help in addressing the probabilities of DSS/DHF incidence in future.
REFERENCES


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Crill WD and Roehrig JT. (2001). Monoclonal antibodies that bind to domain III of dengue virus E glycoprotein are the most efficient blockers of virus adsorption to Vero cells. *J Virol,* **75**:7769-7773.


APPENDICES

Appendix A: Informed Consent Form

CONSENT FORM (Hospital Based Study)

Consent to Participate in a Research Study

PROJECT TITLE: PREVALENCE OF DENGUE VIRAL INFECTIONS AMONG FEBRILE PATIENTS IN MOMBASA COUNTY, KENYA

Name of Clinic/Health Facility: _____________________________________________

Study Number: ______________________ Date of Hospital Visit: ___________

1. PURPOSE: The purpose of this study is to assess the prevalence of dengue virus infection.

I would like to ask you to volunteer and take part in this research project, which will include a minimum of 384 patients.

2. PROCEDURES: If you agree to participate in this study, we will ask you/your child for a small sample of blood if you/your child has high-grade fever of >39.5°C. A blood sample (3 ml) will be taken from the arm (venipuncture). The blood will be used to attempt to identify dengue antibodies.

3. RISK TO PARTICIPANT: Blood will be drawn from your/child’s arm with a needle by an experienced phlebotomist. The risk that you/your child may be injured during collection of blood is minimal, but it is possible that there may be some pain and discomfort when the blood is removed from the arm; afterwards there may be some bruising or swelling and a very small possibility of infection at the site where the blood was collected. You/your child may feel faint when the sample is taken but this is uncommon and the feeling will pass quickly.

4. POTENTIAL BENEFITS: The possible benefit to you/your child from taking part in this study is that the blood samples they tell you/your child have or had dengue infections.

5. COST AND COMPENSATION: There is no cost to you to participate in the study.

6. MEDICAL CARE FOR RESEARCH RELATED INJURY: If you/your child is injured as a direct result of taking part in this research project, you/your child will be given medical care for that injury. This will be given to you/your child at no cost. You/your child will not receive any injury compensation, only medical care. Signing this document does not limit you/your child rights to seek legal remedies through the legal system.

7. SUBJECT CONFIDENTIALITY: All the information related to this project will be confidential. The data may be reviewed by the Institutional Review Committee and Scientific Steering Committee. We will keep them private to the extent legally as possible.

8. VOLUNTARY PARTICIPATION: You/your child can decide not to take part in this study without any negative consequences.
9. POINTS OF CONTACT: If you/your child want to talk to someone about this study or if you/your child have been injured from taking part in this study, please contact Prof. Matilu Mwau (Principal Researcher) of KEMRI at 02027222541 ext 2256/2290

10. CONSENT: THE PROJECT HAS BEEN EXPLAINED TO ME IN A LANGUAGE AND LEVEL I CAN UNDERSTAND. I HAVE BEEN ENCOURAGED TO ASK QUESTIONS ABOUT THE RESEARCH STUDY. MY SIGNATURE BELOW WILL INDICATES THAT I HAVE CONSENTED TO VOLUNTEER TO TAKE PART IN THIS STUDY.

My Name: ________________________________________________________________
Age: ______________________________________  Sex: _________________________
Signature: __________________________________________________________________

It is possible that after we have completed the laboratory tests on your blood samples, there will be some leftover. What do you want us to do with this leftover blood sample? (Initial only one option)

A. _______ After the study is completed destroy all remaining specimens.

B. _______ After the study is complete the remaining specimens can be used for any scientific purpose provided that the scientific purpose is approved by an Scientific Steering Committee and that my specimen will not be identified by name but only by a number.

I also understand that there will be no compensation for the future use of my specimen(s).

If you change your mind at any time, and would like their leftover blood samples destroyed contact Dr. Matilu Mwau (Principal Researcher) of KEMRI at 02027222541 ext 2256/2290.

If the parent or guardian is illiterate, an adult must witness the consent process.

Name of Witness: ___________________________________________________________ Age:____________________
Signature of Witness: _______________________________________________ Date:____________________

Name of Investigator: __________________________________________________________
Signature of Investigator: __________________________________________ Date: ___________________
## Appendix B: Clinical Investigation Questionnaire of Febrile Illnesses

### PREVALENCE OF DENGUE VIRUS INFECTION AMONG FEBRILE PATIENTS IN MOMBASA COUNTY

<table>
<thead>
<tr>
<th>Clinic/health facility name:</th>
<th>Study Number:</th>
<th>Date of hospital visit:</th>
<th>Age:</th>
<th>Sex:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<table>
<thead>
<tr>
<th>Clinic Code:</th>
<th>Village of residence:</th>
<th>Location:</th>
<th>County:</th>
<th>Province:</th>
</tr>
</thead>
<tbody>
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<table>
<thead>
<tr>
<th>Sex: M ( )</th>
<th>F ( )</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Study Number:</th>
<th>Date of hospital visit:</th>
<th>Age:</th>
<th>Sex:</th>
<th>Village of residence:</th>
<th>Location:</th>
<th>County:</th>
<th>Province:</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Marital status:</th>
<th>Occupation:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single ( )</td>
<td></td>
</tr>
<tr>
<td>Married ( )</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Single ( )</th>
<th>Occupation:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Head of the household ( )</td>
<td></td>
</tr>
<tr>
<td>2. Mother ( )</td>
<td></td>
</tr>
<tr>
<td>3. Caretaker ( )</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Have you ever been vaccinated for any Arbovirus infection?</th>
<th>If yes, which one?</th>
<th>If Yes, when:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes ( )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No ( )</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical signs and symptoms:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Fever</th>
<th>If Yes, Duration of symptoms:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes ( )</td>
<td></td>
</tr>
<tr>
<td>No ( )</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Skin rashes</th>
<th>If Yes, Duration of symptoms (in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes ( )</td>
<td></td>
</tr>
<tr>
<td>No ( )</td>
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<table>
<thead>
<tr>
<th>Abdominal pain</th>
<th>If Yes, Duration of symptoms (in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes ( )</td>
<td></td>
</tr>
<tr>
<td>No ( )</td>
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<table>
<thead>
<tr>
<th>Myalgia</th>
<th>If Yes, Duration of symptoms (in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes ( )</td>
<td></td>
</tr>
<tr>
<td>No ( )</td>
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<table>
<thead>
<tr>
<th>Headache</th>
<th>If Yes, Duration of symptoms (in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes ( )</td>
<td></td>
</tr>
<tr>
<td>No ( )</td>
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<table>
<thead>
<tr>
<th>Bleeding diathesis:</th>
<th>If Yes, Duration of symptoms (in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes ( )</td>
<td></td>
</tr>
<tr>
<td>No ( )</td>
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<table>
<thead>
<tr>
<th>List any other symptoms</th>
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<table>
<thead>
<tr>
<th>Name of person completing form:</th>
<th>Signature:</th>
<th>Date:</th>
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Appendix C: KEMRI Ethical Review Clearance

KENYA MEDICAL RESEARCH INSTITUTE

KEMRI/RES/7/3/1
August 23, 2011

TO: DR. MATILU MWAU (PRINCIPAL INVESTIGATOR)
DIRECTOR, CIPDCR
BUSA

Dear Sir,


Reference is made to your letter dated August 22, 2011.

We acknowledge receipt of:
  a. The Revised Study Protocol

This is to inform you that the Ethics Review Committee (ERC) finds that the issues raised at the initial review have been adequately addressed. Consequently, the study is granted approval for implementation effective this 23rd day of August 2011.

Please note that authorization to conduct this study will automatically expire on August 21, 2012. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by July 10, 2012.

Any unanticipated problems resulting from the implementation of this protocol should be brought to the attention of the ERC.

You are also required to submit any proposed changes to this protocol to the ERC to initiation and advise the ERC when the study is completed or discontinued.

You may embark on the study.

Sincerely,

Christine Wasunna,
FOR: SECRETARY,
KEMRI/NATIONAL ETHICS REVIEW COMMITTEE

In Search of Better Health